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ULTRASTRUCTURAL ASPECTS OF RAT COLON:

Morphometric Comparison of Cell Interfaces in Normal And Potassium Loaded Rats

CAROLINE RIX TAYLOR

1978

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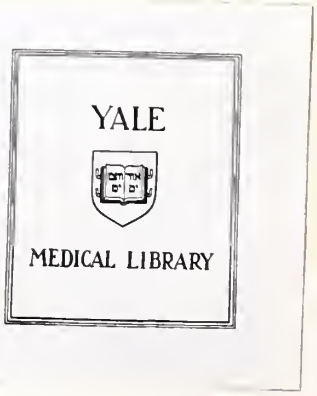

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ULTRASTRUCTURAL ASPECTS OF RAT COLON: MORPHOMETRIC COMPARISON OF CELL INTERFACES IN NORMAL AND POTASSIUM LOADED RATS.

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A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the degree of Doctor of Medicine, 1978.

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INTRODUCTION.

Epithelia line and protect surfaces and have important roles in the secretion and absorption of ions and electrolytes. Recent investigations have focused upon colon as a model for a potassium transporting epithelium which in many ways is analogous to distal renal tubule. The colon and kidney can both adaptively increase the rate of potassium secretion, associated with increases in transmural potential difference and activity of Na-K-ATPase.

The purpose of this study is to investigate various morphologic characteristics of colonic epithelium by electron microscopy, and to measure the intercellular membrane surface area by a morphometric technique. The intercellular membranes have an important role in ion transport as, in colon, they contain Na-K-ATPase.

SECTION 1: ULTRASTRUCTURAL ASPECTS OF COLONIC EPITHELIUM.

The function of epithelium is to line surfaces and to separate anatomically and physiologically the internal environment from the external, thereby maintaining the integrity of the organism. Colonic epithelium is an anatomical barrier to the gut lumen and its contents, but also selectively transports water and electrolytes from lumen to serosal capillaries or vice versa via the epithelial cells.

Microscopically, the colon is composed of simple columnar epithelium with brush border. This epithelial surface facing the lumen is relatively smooth, although scanning electron microscopy reveals surface convolutions subdividing groups of epithelial cells[1]. The absorptive, or principal cells are the predominant cell type at the epithelial surface; they are interspersed with goblet cells, the major cell type in the crypts of Lieberkuhn.

Histochemical studies have revealed certain differences between cells of proximal and distal colon; ABH iso-antigens have been demonstrated in proximal but not distal colon[2]. There is a predominance of acid muco-substances in the large intestine, with an increased amount of sulfo-mucins in distal colon and a preponderance of neutral muco-substances in the proximal colon, decreasing distally[3].

Early electron microscopy studies reviewed colonic epithelial ultrastructure[4,5,6]. The principal absorptive cells are the subjects of this present study (Figure 1.1). They consist of tall simple columnar epithelium, with basilar or centrally located nuclei and luminally oriented microvilli. The dimensions of these microvilli in man are 2μm x 0.25μm, and they are covered by a filamentous coat extending 2μm into the gut lumen[7]. This coat is rich in polysaccharides, which are bound to the cell membrane[8]. Golgi membranes are prominent supranuclearly. The Golgi apparatus has recently been shown in rat descending colon to complete the glycosylation of glycoprotein incorporated into plasma membrane and cell coat[9]. The paranuclear and basilar regions con-

tain mitochondria and rough endoplasmic reticulum.

The junctions between cells are well defined[1]. Intestinal absorptive cells provide excellent examples of tight junctions. Desmosomes are prominent in human colon[10]. The lateral cell walls interdigitate with those of adjacent cells with marked interlacing[11]. These complex interdigitating folds have been thought to hold cells together[8].

Basal infoldings are commonly found in cells such as clear cells of sweat glands and distal convoluted kidney tubule, which are actively engaged in active transport of fluids and ions[12]. Recently Na-K-ATPase, which is involved in the active absorption of sodium and excretion of potassium[13] has been demonstrated along the baso-lateral membranes of epithelial cells of intestinal mucosa[14,15,16].

The epithelial cells overlie a basement membrane. Collagen fibres are incorporated into the supporting stroma below epithelial basement membrane but are absent at crypt bases[17]. Cell regeneration occurs at the crypt bases, with migration of cells toward the lumen. Dying absorptive cells are found midway between glands and are characterised by decreased electron density and loss of microvilli, and later cell contents[7].

Ultrastructural studies have also demonstrated other cell types, such as lymphocyte-like cells[18], argentaffin cells in crypt bases which contain electron dense material consisting of amine and polypeptide granules[1,7,19], and multivesicular cells with large microvilli[20]. Goblet cell ultrastructure has been well described by Florey[21] and Pfeiffer[1].

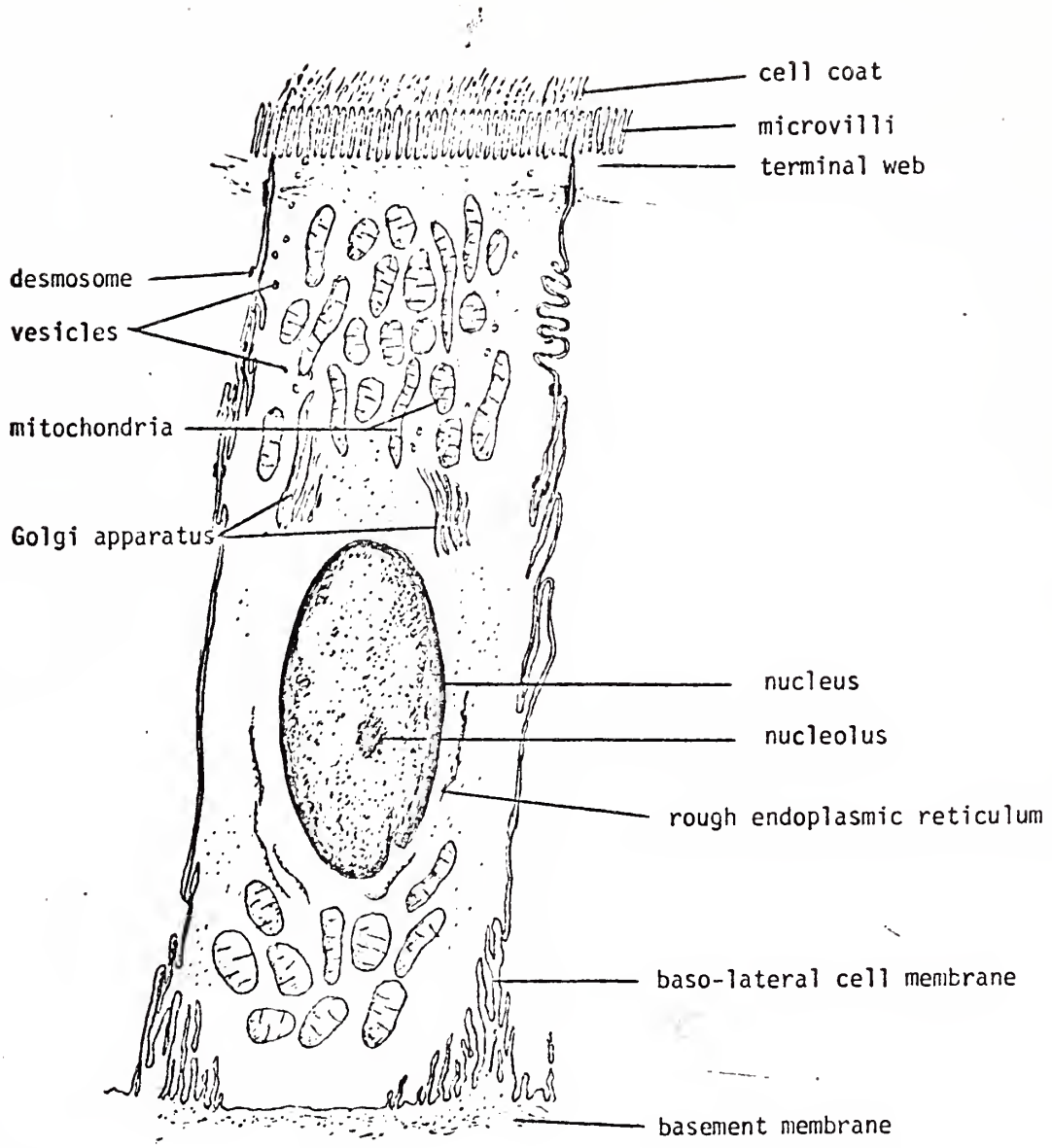


Figure 1.1. Schematic representation of colonic absorptive cell ultrastructure

SECTION 2: PHYSIOLOGY OF THE COLON.

i. ORIGIN OF TRANSMURAL POTENTIAL DIFFERENCE.

In rat colon, a transmural lumen negative electrical potential difference exists which is estimated to range between 8 to 27mV under resting conditions[22 - 25]. In man, a PD of 30 to 40mV has been described[26]. There is little variation of PD along the length of the colon, human or rat[24,27]. This PD, unlike that measured in small intestine (where it is usually less than 10mV) is unaffected by the presence of glucose or amino acids in gut lumen[26].

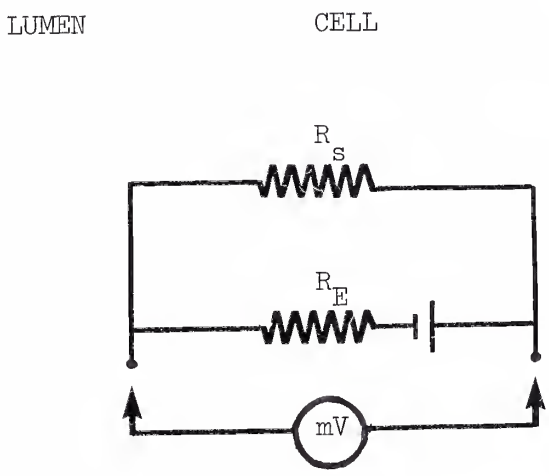
The PD is, however, affected dramatically by certain hormones. Sodium depletion provokes an increase in aldosterone secretion by the adrenal gland [28,29]. The electrical PD markedly increases in rats treated this way, increasing to between 20 and 56mV, with a greater increase in distal colon compared to proximal[30].

Adrenalectomy is associated with a fall in PD to below 5mV[23]. Twenty-four hours after adrenalectomy, the potential difference may fall to 20% of control value, which in one experimental series, was 25 $\pm$ 3.4mV lumen negative[25].

Aldosterone given intravenously to adrenalectomised rats (1µg/100gm) causes a rise in potential difference that is greatest in distal colon, with an onset of action two hours following injection. The PD remains elevated provided that replacement continues[23]. Other authors, however, have not obtained PD elevations in rats given aldosterone (10µg/100gm), but noted a return to normal PD when dexamethasone (10µg/100gm) was administered[25,31]. Edmonds and Marriot[23] noted a minor but significant effect of cortisol (40µg /100gm) on distal rat colon PD. Methylprednisone and DOCA have also been shown to increase transmural PD in colon, but while MP affects jejunum and ileum also, DOCA is specific to colon[32].

The transmural potential difference is set up by the difference in ion concentrations across the colonic epithelium secondary to the different

mobilities of these ions in solution. Osmotically induced PDs have not been demonstrated in colon[26], although they have been described in gall bladder and intestine.



$$V = ER_s / R_s + R_E$$

where E = EMF
V = transmural PD
R_E = int. resistance
R_s = shunt resistance

Figure 2.1 Scheme from Edmonds[26] to illustrate properties of transmural potential difference in colonic epithelium.

Figure 2.1 shows components of an electrical model to describe transmural PD. The measured PD is less than that of the transport generated EMF because of the presence of a shunt R_s . There is evidence that chloride is the main ion involved in the shunt current. The transfer PD is dependent on active transport; in vitro experiments have shown that it can be reduced or abolished by anoxia, metabolic inhibitors, or ouabain[33]. This model is of course simplistic, and does not describe the individual contributions of separate cell elements and ions to the transmural PD. For a lucid description of the difficulties encountered in characterising ionic movements across epithelia, the reader is referred to an article by Macknight[34].

ii. SODIUM TRANSPORT IN THE COLON.

Sodium is probably the main factor in the transfer PD of colonic mucosa[35] as it is the major cation actively transported against the electrochemical gradient. In man, the normal colon absorbs 1350ml water, 200mEq sodium, 150 mEq chloride, and 60mEq bicarbonate per day with no net movement of potassium[36].

Estimates of the electrochemical gradient have been made; colonic mucosa can absorb sodium from luminal concentrations of 15mEq against a gradient of 40mV[36]. More recently, Bastl et al[37] documented sodium absorption against a maximal gradient of 60mV. Yau and Maukhlouf[38] examined both ascending and descending rat colon and found active absorption of sodium in both segments as determined by net flux and chemical gradients, although the ascending colon absorbs more sodium[38,39]. The passive permeability to ascending colon (and rat ileum) is two to three times higher than in descending colon[22].

Sodium transport does not appear to be related to potassium secretion[40]. Water movement is however passive and parallels sodium movement, even when secretion of sodium is stimulated by bile salts. Chloride transport has been considered passive[22], but it can be absorbed from luminal solutions in a concentration as low as 25mEq/l[41], and more recently it has been suggested that net chloride transport is coupled to sodium transport in a neutral electrogenic mechanism[42]. It may be exchanged for bicarbonate ion, as in ileum; bicarbonate is secreted into colon against electrochemical gradients[36]. The relationship of secretion to carbonic anhydrase, an enzyme present in large quantities in cecum and descending colon, is not clear[43].

Although transport of sodium is dependent on the Na-K-ATPase pump, entry of sodium into cells is also affected by adenyl cyclase in the cAMP system. while cholera enterotoxin has been shown to cause water and electrolyte secretion in small bowel via an increase in cAMP synthesis, bile acids act in colon in an analogous fashion[42]. By blocking or enhancing this system it is possible to alter sodium and water secretion; for example, theophylline stimulates cAMP and electrolyte secretion via inhibition of phosphodiesterase, and propranolol inhibits the stimulation induced by deoxycholic acid[44] by decreasing adenyl cyclase.

iii. POTASSIUM TRANSPORT IN COLON.

It has been suggested that potassium transport is a passive process[46]

which occurs in response to electrical gradients in gut - in colon, a large electrical PD is present[36] generated by active transport of sodium. In vivo perfusion studies have shown that potassium is secreted by colonic mucosa, with net absorption occuring when the perfusate contains more than 15mEq/l[41].

Potassium transport appears to be mainly independent of the rate of sodium absorption and luminal concentration[40] and there is no direct evidence to suggest that there is a direct exchange of sodium for potassium, as had been originally suggested[47]. A fixed relationship of sodium to potassium uptake is unlikely to exist[24]. However, the potential difference appears to be closely related to potassium secretion[25] and it may act as a driving force for potassium movement. Potassium secretion in colon is not flow dependent[37].

There is some disagreement over a postulated active component to potassium transport. Edmonds[48] felt that potassium efflux might be a function of diffusion and active transport, with efflux being due to simple diffusion. It has also been suggested that measured fecal concentration in man cannot be the result of simple diffusion, with passive distribution of potassium across the epithelium, as this would require the presence of a transepithelial PD of some -80mV in distal colon[40].

Part of the difficulty in interpreting conflicting results may arise from the fact that colon does not behave in a uniform physiologic manner. For example, though distal colon transports much less in absolute terms than proximal colon, it is capable of creating considerable ionic gradients[40]. Recent investigations have shown that differences exist between potassium transport in proximal and distal colon. Yau and Mauhklouf[38] found active transport in descending colon occuring against both electrical and chemical gradients, but zero net flux in ascending colon, where potassium was distributed close to the electrical equilibrium. A recent study, however[49], determined that active transport of potassium is found in proximal but not distal colon under conditions of steady state potassium concentration with no net fluid movement.

iv. PATHWAY OF ELECTROLYTE MOVEMENT IN COLON: TRANS VS INTERCELLULAR

It is felt that sodium and potassium movement across colonic epithelium is transcellular, rather than via intercellular channels[50]. There may be an anatomic basis for this; colonic epithelium, like that of small intestine, is joined by so-called tight junctions. It has become apparent that all such junctions do not possess the same properties. In gall-bladder, small intestine and renal proximal tubule, for example, the intercellular route is the major pathway for electrolyte fluxes[51]. These epithelia have 'leaky' junctions, characterised by a low electrical resistance, low transmural PD, and high osmotic water permeability, and they transport isotonic fluids. Only shallow gradients can be set up by active solute transport and solvent drag in lateral cell spaces. Water movement affects absorption of potassium, a passive process occuring between, not through, epithelial cells[34].

The colonic tight junction, in contrast, is able to maintain a steep concentration gradient, and is not penetrated by lanthanum[52], an ion which permeates gall-bladder and ileal epithelium[53]. In comparison to ileum which has a PD below -10mV, and whose sodium transport is influenced by glucose and amino acids, the colon maintains a PD of up to 50mV and possesses an active transport system which resembles that of distal renal tubule: its enzymatic properties, electrical properties and net flux changes are very similar[24].

Some controversy does appear to exist over this classification. Colonic epithelial junctions, while not as leaky as small intestine, are not as tight as toad bladder epithelium[34]. Leaky epithelia have been noted by Schulz and Frizzel[54] to have negative intracellular PDs with respect to lumen and have described this condition in colon. They therefore believe that on this basis transepithelial potassium movements are entirely diffusional and probably restricted to paracellular pathways. However, Schulz[54] notes that the finding of an electrically negative interior in a leaky epithelium may also be consistent with an EMF across the membrane oriented in the same direction as that

measured in tight epithelia, where the intracellular PD is positive with respect to lumen.

v. THE MECHANISM OF ELECTROLYTE TRANSPORT IN COLON: THE ROLE OF NA-K-ATPase.

Na-K-ATPase is an enzyme related to active sodium absorption and potassium excretion[13] and has been demonstrated in brain, muscle, nerve, kidney, and gut, including colon[55,56]. It is sited along the baso-lateral membranes of colonic mucosa[55] and acts as a ouabain sensitive sodium pump at the serosal membrane[57]. As colonic epithelium is analogous to distal tubule in many of its characteristics, including potassium handling, the model for Na-K-ATPase action in distal renal tubule will be briefly described. The tubule cell is electrically asymmetrical with two pump leak systems located in series[24]; the Na-K-ATPase is on the baso-lateral membrane. It has two species of interaction sites on the membrane[58]. The inner site has a high affinity for sodium, the outer for potassium. Uptake of potassium is regulated at the serosal side. It diffuses out of the cell across the partly depolarised membrane, where the second pump leak system is situated. This opposes efflux of potassium via an active absorptive process[24]. Figure 2.2 illustrates this concept.

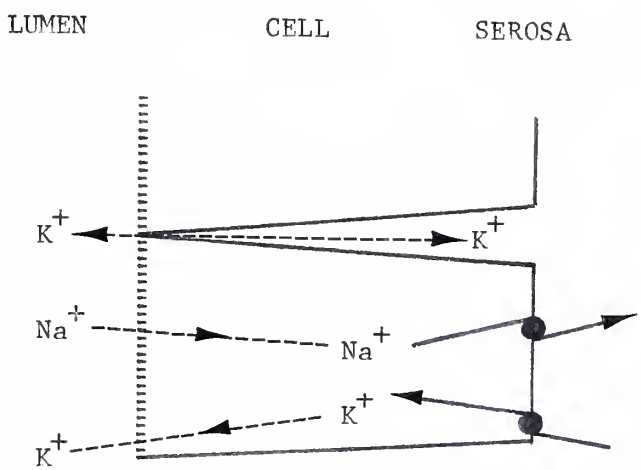


Figure 2.2 A model to illustrate ion movement across colonic epithelium. Diffusion pathways are represented by broken lines and active transport by solid lines. Sodium movement is transcellular. Potassium is transported transcellularly but may also diffuse intercellularly.

The rate of sodium transport across the serosal cell membrane is a function of the intracellular concentration, which is in turn responsive to the rate of sodium entry across the luminal cell surface[59]. There is evidence that in some epithelia, the intracellular concentration of ions can affect the movement of sodium through channels in the luminal face of the cell- in frog skin epithelium, an increase in intracellular sodium, and a decrease in intracellular potassium reduce the availability of sodium channels in the mucosal face. This explains why ouabain also inhibits influx through the mucosal face: it does not occur simply because of a decreased gradient as the intracellular pool fills up[60]. A fixed relationship with potassium uptake is unlikely to exist[24], but potassium activates Na-K-ATPase at low concentrations and inhibits it at high ones, when it competes with sodium at the intracellular site[61]. Net potassium movement depends whether the cell is adapted to excretion or absorption of potassium.

vi. THE RATIONALE FOR THE CURRENT INVESTIGATION OF COLONIC EPITHELIAL CELL ULTRASTRUCTURE AND LATERAL CELL MEMBRANES.

Although there is uncertainty over the actual mechanism of potassium secretion, there is no doubt that like kidney, the colon can handle potassium in an adaptive way. Potassium secretion is closely linked to the activity of Na-K-ATPase in kidney, and increases following dietary loading[62] with an accompanying rise in PD of the distal tubule[63]. Renal insufficiency also increases the activity of renal Na-K-ATPase, as this also increases the excretory load of potassium per unit of remaining renal mass [64,65].

The colon responds to dietary depletion of potassium by decreasing secretion virtually to zero[37] and increases secretion in response to chronic dietary loading[26]. Another manipulation which increases the excretory load of potassium to the colon is surgically induced renal insufficiency, or renal failure. For example, stool potassium is increased in patients with severe chronic renal failure, suggesting adaptive excretion of potassium[66], and in an exper-

imental model, 70% renal ablation in rats produces increased net colonic potassium secretion with an accompanying increase in net sodium absorption[25].

These increases in colonic secretion of potassium are accompanied by increases in colonic transmural PD[24,25]. Chronic dietary potassium loading has been shown to increase PD to a maximum value of 50mV, measured at 6cm from the anus in rats[24]. Na-K-ATPase appears to increase its activity in addition [24,25,66] with no demonstrable increase in jejunum[24], muscle, brain or liver[62]. Furthermore, there is no effect on secretion of sodium, water, chloride or bicarbonate[24].

Because colon possesses some of the same characteristics as kidney with regard to potassium excretion, this organ is increasingly being used as a model to study ion transport. Ultrastructural observation of epithelial cells from rat colon after chronic dietary potassium loading revealed marked hyperplasia of the intercellular membranes[67]. Since Na-K-ATPase activity is found in the baso-lateral cell membranes of colon it was postulated that this might represent a morphologic correlation, on an ultrastructural scale, with the pre-viously described biochemical and physiological changes associated with chronic dietary potassium loading.

It was therefore decided to quantitate this observation by making morphometric measurements of the intercellular colonic membranes from electron micrographs in order to examine the difference between proximal and distal colon in normal control animals and in potassium loaded rats. Also, the general char-acteristics of colonic cells were examined in order to compare proximal and distal colon in the light of physiologic data which suggests that there are functional differences between these cells.

SECTION 3: MATERIALS AND METHODS.

i. THE QUANTITATIVE MEASUREMENT OF CELL INTERFACES IN RAT COLON EPITHELIUM

Morphometry is used to make objective comparisons between cell populations where morphological parameters are the basis of study and where only small quantitative differences may exist. This statistical technique enables organelle structure to be systematically estimated. Quantitative relationships exist between the average dimensions of a large number of organelles and those of their profiles on ultra-thin section[68]. The aim of this study was to examine and compare the intercellular membranes of colonic absorptive cells in control and potassium loaded rats. Loud[69] described a method for quantitating endoplasmic reticulum by measuring the length of its irregular profile on thin sections. This technique has been used successfully to measure lateral cell walls in rabbit proximal nephron[70] and has been adapted for use in this study.

Six adult male Sprague-Dawley rats (three controls and three potassium loaded) were fed a diet of powdered Purina chow, containing 25mEq KCl/100gm. In addition, the potassium loaded rats were fed supplemental potassium, 275 mEq KCl/100gm Purina chow, for one week. The animals were killed by perfusion with quarter strength Karnovsky's solution under Inactin anesthesia[71]. This solution contains paraformaldehyde and glutaraldehyde buffered to a final pH of 7.2 with sodium cacodylate in 0.6M sucrose. The method of tissue preparation was standardised, as this enables valid comparisons to be made[68].

The colon was then isolated and divided into proximal and distal portions. Coded segments were then embedded in epoxy resin. The blocks were then trimmed and thick sectioned with a glass knife, and stained with methylene blue for examination under a light microscope. The suitability of the tissue for ultra-thin section was determined by several criteria. Tissue was excluded if it appeared grossly shrunk, or was obliquely sectioned. It was necessary to select epithelium with parallel arrays of absorptive cells, containing visible

nuclei. Ultra-thin sections were then cut with a microtome (UltratomeIII) using a diamond knife. They were stained with 2% uranyl acetate solution and 2% lead citrate solution and then examined under a Zeiss 10 electron microscope. The sections were first scanned at low magnification (x2500) until suitable areas were found for photography. Parallel arrays of absorptive cells, with visible nuclei, intact basement membranes, and abundant microvilli were considered suitable provided there was no section compression.

The key to the analysis of photomicrographs is a grid which consists of a transparent overlay with evenly spaced perpendicular and parallel lines of length L separated by a distance D that can be applied to photographs of the sections, in a number of different orientations. Lateral cell membranes appear on cross section as convoluted lines, and a measure of the length of their profiles can be calculated from the frequency with which they intersect the sampling line of the grid (the number of crossings, C). From this the membrane profile concentration - the average number of microns of intercellular membrane per square micron of cytoplasm in the unmagnified cross section - can be estimated (for derivation see Loud[69]).

The membrane profile concentration = ΠCM/2000L, where C = no. crossings M = overall magnification, L = mm sampling lines crossing the cell cytoplasm in one direction. The membrane surface concentration can be simply derived from this as the average number of intercepts per unit length of a random line drawn through a three dimensional structure is exactly half the true ratio of surface to volume[72]. MSC = $(4/\Pi)$MPC or CM/500L.

The actual area of structural interface is in fact twice this as the cell interface has two sides, thus

$$MSC = CM/250L\ \mu m^2/\mu m^3.$$

This formula is valid for comparative studies, but may not be a true estimate of cell membrane surface area, as obliquely cut sections of membranes

are not visualised[69]. For the measurement of rat colonic intercellular membranes, sections were initially photographed at magnifications of 2500 to 10000, and were enlarged a further 2.5 times on to 10" by 8" prints. By trial and error it was determined that an EM magnification of 4000, enlarged to x10000, was acceptable, as this was the lowest magnification at which details of the intercellular membrane were consistently visible. At a higher magnification, section area was limited so that sampling was no longer representative of the whole tissue. At a magnification of 4000, it was often not possible to include an entire cell and so data from upper and lower halves of a cell were combined as recommended by Hyatt[68].

The grid lines were separated by 5mm in order that the number of crossings exceeded 400 per micrograph in any orientation. This limits the standard error to less than 5%, as the statistics resemble those used in hemocytometers, where the standard error is equivalent to the square root of the number of counts[73]. Thus an acceptable error is 20 in 400 crossings, or 5%[69]. The coincidental alignment of membranes may also increase the error, but this was limited by using a grid with perpendicular and parallel elements and applying it to the micrograph at orientations of $0/90^{\circ}$ and 45°.

There are no fixed rules determining sample size, but because this is a statistical technique adequate numbers had to be obtained to represent the tissue. In this case. There were three animals in each group and ten blocks per animal per colon segment. One set of sections was ideally obtained from each block, and 5 - 10 representative areas were photographed per section. This provided approximately 20 micrographs per group suitable for analysis.

The material from each animal was initially examined "blind" to exclude observer bias, and results were combined before the final analysis was made. The four groups (proximal and distal colon of control and potassium loaded rats) were analysed in standard statistical fashion so that mean membrane surface concentrations could be compared.

ii. Calculation of surface area of microvilli in relation to

Luminal cell surface area .

On a photomicrograph, there are x microvilli per y μm luminal cell surface in one plane, ie there are x/y microvilli per μm, or $(x/y)^2$ microvilli/μm^2. If it is assumed that the microvilli are almost in contact with each other, then the diameter of each microvillus is y/x μm, or radius r is y/2x μm. As microvilli resemble cylinders with round tips, equivalent to a half sphere, then the surface area of one microvillus is represented by: $2 \pi r (h+r)$, where SA cylinder = $2\pi rh$ and SA half sphere = $2\pi r^2$. (r is radius and h is height of a microvillus).

Thus, as there are $(x/y)^2$ microvilli per μm^2, the surface area of microvilli in one square micron of luminal cell surface is:

$$(x/y)^2 (h+r) 2\pi r$$

or, substituting for r,

$$\pi hx/y + \pi/2 \ \mu m^2 \text{ microvilli per } \mu m^2.$$

Measurements from each of the four groups, ascending and descending colon in control and potassium loaded rats, were made from photomicrographs at a magnification of x10000. The number of microvilli within a five centimetre distance (measured along the bases of the microvilli) were counted. These were then statistically analysed in order that density, height, and estimated surface area in relation to luminal cell surface could be compared.

iii. Measurement of further parameters in the colonic absorptive cell

In addition to measuring membrane surface concentration, data were obtained for height and width of colonic cells. Photomicrographs were taken at a magnification of x2500 in order to enable entire cells to be visualised. Cell height was directly measured on undistorted absorptive cells in the long axis of the cell, through the nucleus. Cell width was measured at the level of the nucleus in a plane perpendicular to the long axis. These sample populations were then statistically compared.

COLON MORPHOLOGY: ULTRASTRUCTURAL STUDY OF PROXIMAL AND DISTAL COLON.

The proximal colonic absorptive cell is tall and slender with a basally oriented nucleus (fig 4.1). Mean cell height is 36.8 ± 1.09 (SEM) and width measured through the nucleus is 5.0 ± 0.18 (all units in microns). The distal absorptive cell is shorter and narrower (figs 4.2 and 3). The mean cell height is 21.4 ± 0.76 μm and width 4.0 ± 0.17 μm.

A number of distinctive features may be readily appreciated. The nuclei of proximal absorptive cells are ovoid (fig 4.1) with occasional slight infolding. They contain well defined nucleoli and electron dense chromatin oriented peripherally beneath the nuclear membrane. In contrast, the distal cell nuclei are markedly invaginated, containing deep clefts which are not apparent on examination with light microscopy (figs 4.2 and 4.3). There is a marked quantity of electron dense nuclear chromatin peripherally.

Mitochondria are abundant in both proximal and distal colonic absorptive cells, packing the apical and basal portions (figs 4.1,2 and 4). These organelles have different morphology in proximal and distal colon; those occupying proximal colonic cells are often elongated and slender (figs 4.1,13 and 4.14). Those in distal colon are more globular (4.7,15); thus mitochondria appear to reflect overall cell morphology.

In addition to rough endoplasmic reticulum and ribosomes scattered throughout the cell substance, there is a well developed Golgi apparatus in each cell, consisting of six or seven stacked parallel membranes with lateral vesicles (4.3,15). Many other coated vesicles are seen scattered through the apical aspect of the cell (figs 4.7,11,12). They occasionally contain electron dense inclusions (4.16), and can be observed apparently fusing with the lateral cell membranes (4.15,17) and with the cell luminal membrane between the bases of the microvilli (4.17,18).

Abundant microvilli line the luminal cell surface. Those in proximal col-

on seem slightly shorter and blunter (figs 4.14,16,18) than their distal colon counterparts (figs 4.15,17,19). The microvilli contain microfilaments (figs 4.16,18) which extend into the terminal cell web (4.8). Extending from the microvillus surface are fine threads which constitute the cell coat (fig 4.16,17). Attached to these threads are electron dense particles seen clearly in figure 4.7 and at a higher magnification in figure 4.19.

The height and number of microvilli appear to decrease with cell age; note for example the dying absorptive cell in figure 4.8 which has lost much of its electron density and most of its microvilli.

The goblet cell is more frequent in the epithelium of distal colon and was in fact excluded from morphometric analysis. It contains electron dense cytoplasm, and abundant rough endoplasmic reticulum (fig 4.7) and discharges mucus into the gut lumen (figs 4.8,15). Other cell types, such as the argentaffin cell (fig 4.5) and ciliated cells (fig 4.6) were also noted.

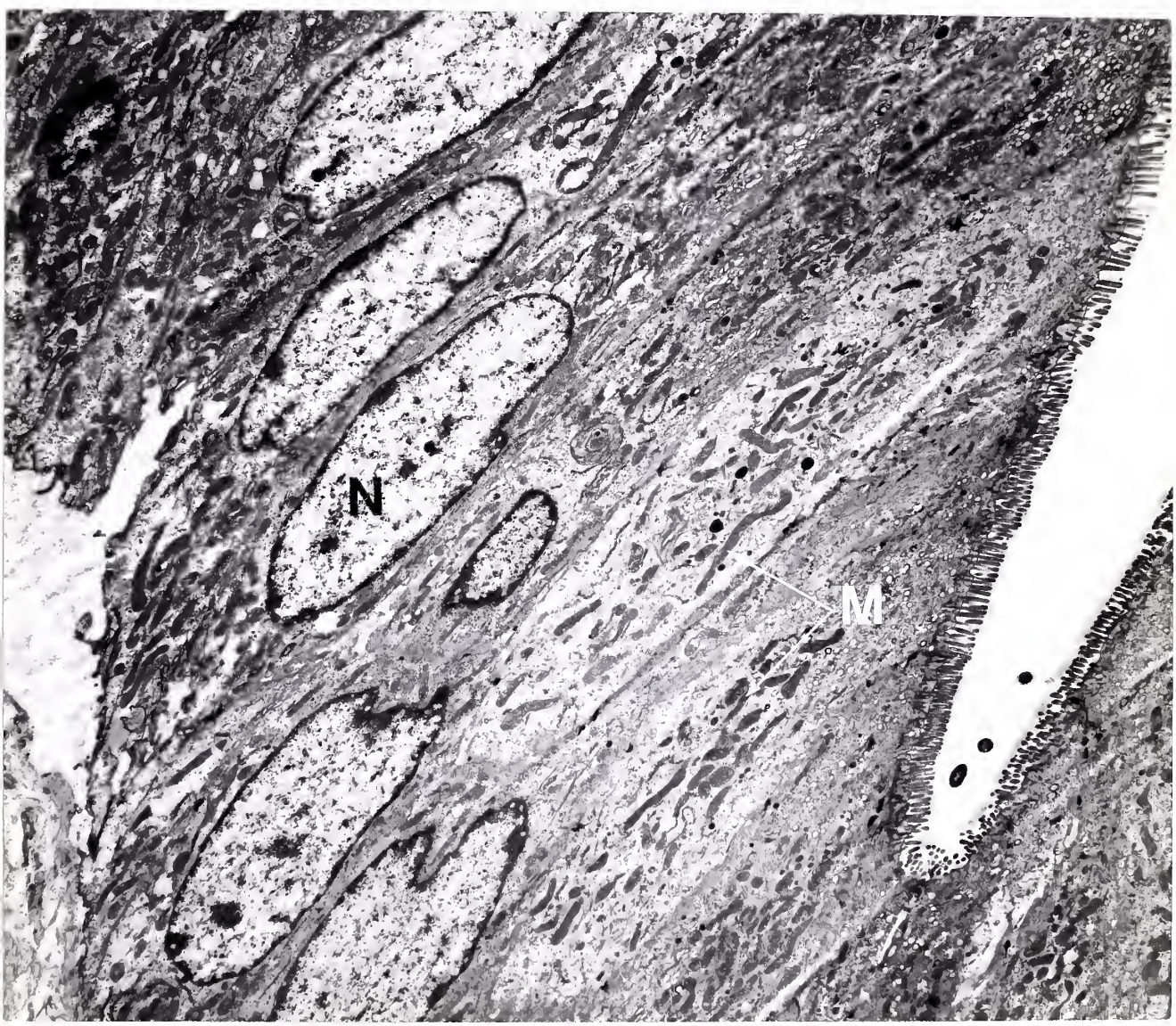


Figure 4.1. Absorptive cells of rat proximal colon x5000

Note elongated nuclei (N), mitochondria (M).

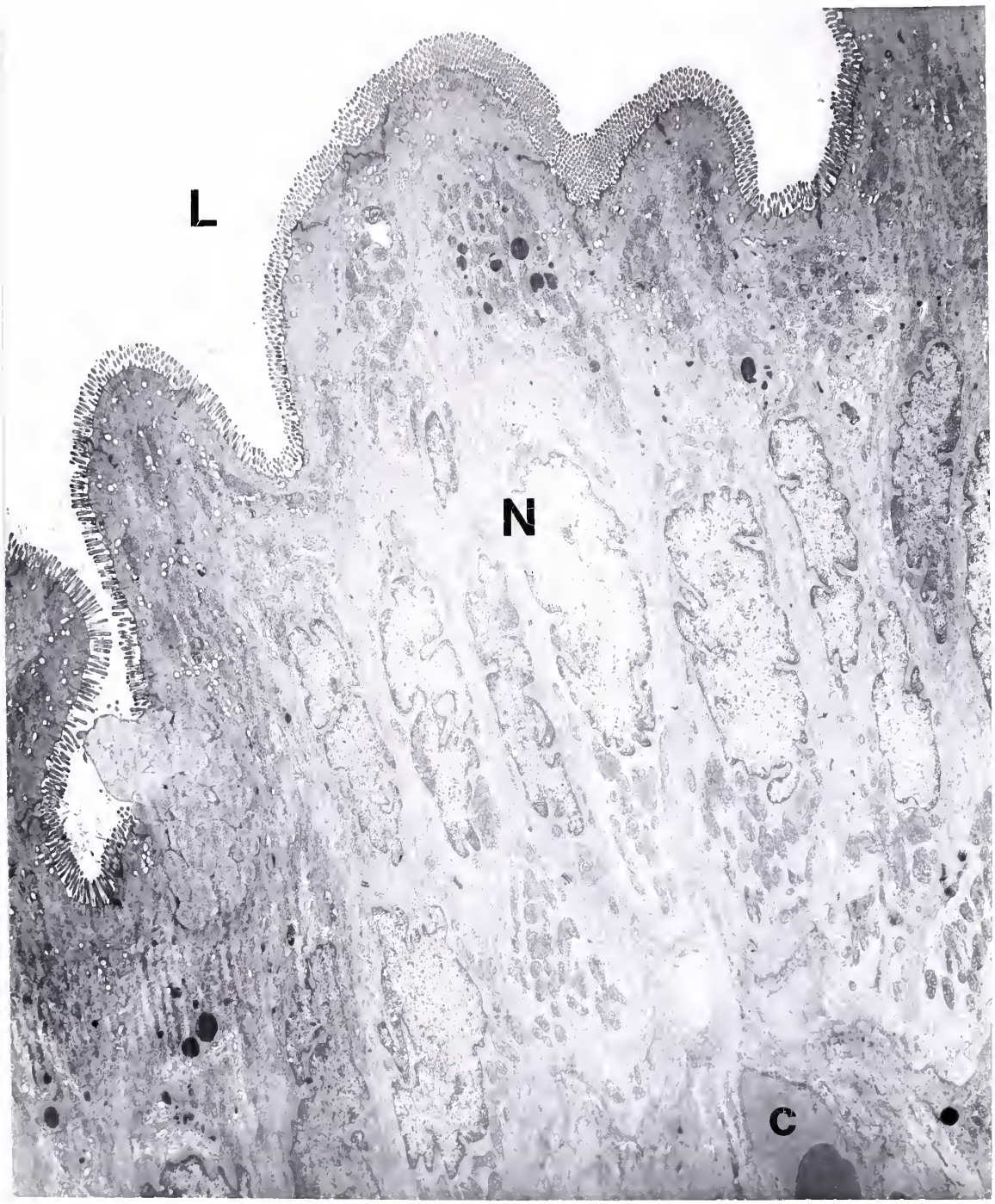


Figure 4.2. Absorptive cells of rat distal colon x5000.

Note invaginated nuclei (N) and apical and basal mitochondria. Gut lumen (L), capillary (C).

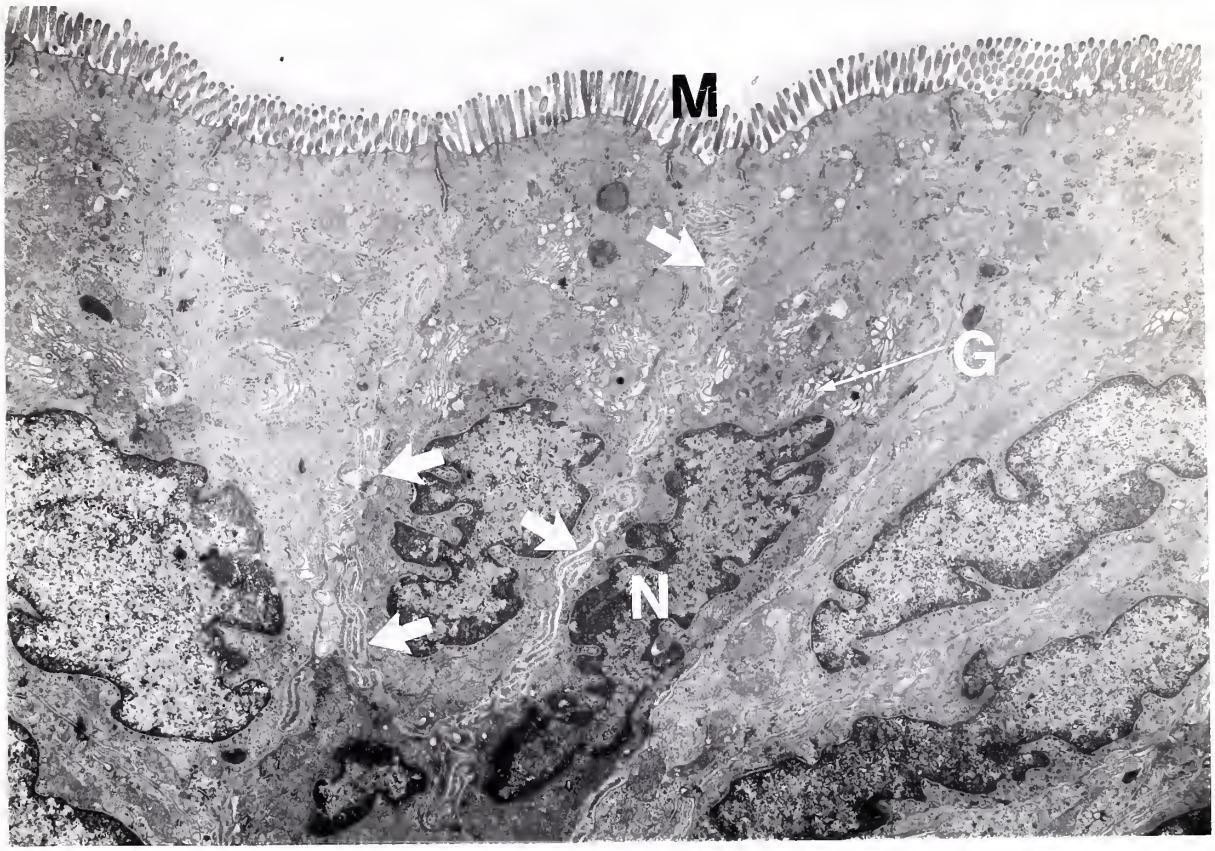


Figure 4.3. Absorptive cells of distal rat colon x8000.

Invaginated nuclei (N) intercellular membrane (arrowed) Golgi apparatus (G) microvilli (M).

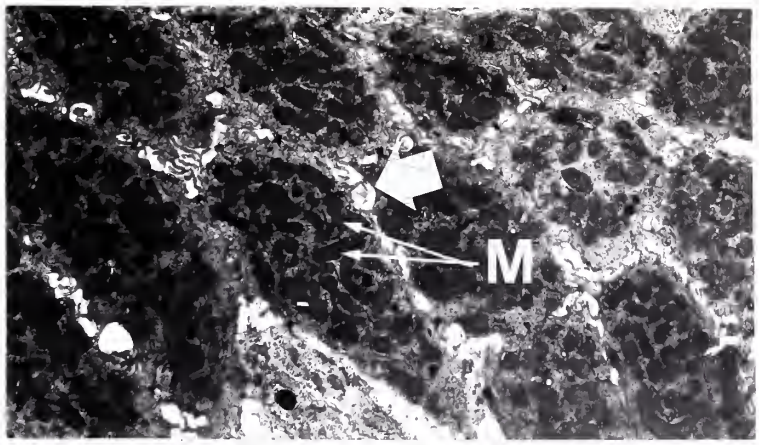


Figure 4.4. Transverse section of absorptive cells of proximal rat colon, apical portion x8000. Mitochondria (M) intercellular spaces (arrowed).

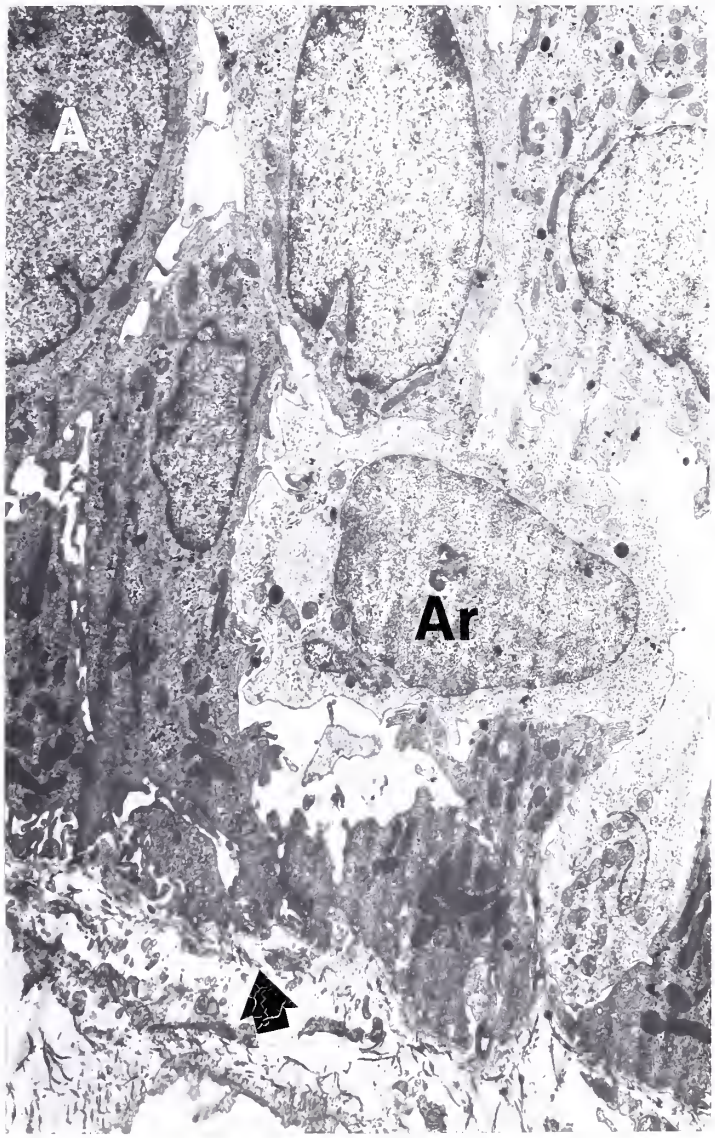


Figure 4.5. Proximal rat colon, distal portion x10000. Absorptive cells (A) and argentaffin cell (Ar) basement membrane (arrowed).

Figure 4.6. Distal rat colon x8000. Absorptive cells (A) ciliated cell with prominent cilia (C).

Figure 4.7. Distal rat colon (potassium loaded animal) x10000. Absorptive cells (A) Goblet cell (G) microvilli (m) cell coat (c) gut lumen (L) intercellular membranes (arrowed).

Figure 4.8. Proximal rat colon (potassium loaded) x5000. Note goblet cell (G) discharging mucus into gut lumen (L) and very prominent rER (arrowed), dying absorptive cell (A) has decreased microvilli (m) and contents are less electron dense.

SECTION 4:ii. QUANTITATIVE DIFFERENCES BETWEEN CELLS OF PROXIMAL AND DISTAL COLON.

In addition to the qualitative differences noted in the previous section, a number of parameters of absorptive cell dimensions were measured. Cell height was measured in proximal and distal colon and the results compared (Table 4.I). The mean cell height in proximal colon is 32.5 ± 1.09μm (SEM). In distal colon, mean cell height is 21.4 ± 0.76μm (SEM). There is a significant difference between the two populations ($p < 0.001$).

Cell width in proximal colonic absorptive cells (Table 4.II) is 4.99 ± 0.319μm (SEM). In distal colonic epithelium, mean width is 3.96 ± 0.174μm (SEM), and this differs significantly from proximal colon ($p < 0.001$).

The number of microvilli per square micron of luminal cell surface was calculated (Table 4.III). In proximal colon, there were 49.6 ± 2.48 (SEM) microvilli per square micron, each with a mean height of 0.8 ± 0.06μm. In distal colon, there were 82.5 ± 5.44 (SEM) microvilli/μm^2 with a mean height of 1.12 ± .039 (SEM) μm. ($p < 0.001$ in each of the two groups, see table 4.IV) Thus there are 66% more microvilli per μm^2 in distal colon, each 40% taller. When surface area is estimated, it is found to be 72% greater in distal colon. In proximal colon, the figure obtained was 19.32 ± 1.52 (SEM)μm^2 microvilli per μm^2 luminal cell surface, and in distal colon, 33.2 ± 1.04 $\mu m^2/\mu m^2$ ($p < 0.001$).

Microvilli in the potassium loaded groups of animals were also examined. There was no significant difference in density of microvilli, height or estimated surface area, between the potassium loaded rats and controls (Table 4. IV). There are 68% more microvilli per μm^2 in distal colon, where they are also 27% taller. Surface area of the microvilli in distal colon in relation to the luminal cell surface is 61% greater than in proximal colon.

A relationship of microvillus surface area in relationship to cell volume may be obtained by dividing the mean estimated luminal surface area by the

mean cell height. In this way, an estimate of luminal cell surface area in relationship to cell volume is obtained. For proximal colon, this is 0.59μm^2 per μm^3, and in distal colon, 1.55$\mu m^2/\mu m^3$. In the potassium loaded rats, these ratios are calculated as 0.62$\mu m^2/\mu m^3$ and 1.52$\mu m^2/\mu m^3$ respectively. These values may not be compared statistically but are of interest when the ratio between luminal surface area and intercellular membrane surface concentration is considered (vide infra).

CELL HEIGHT

CELL	DISTAL	CELL	PROXIMAL
08798:5D4	29.6	09310:5P15	16.8
	27.2		15.5
	24.0		16.5
	20.0	08723:1P4	36.0
	19.2		36.8
	19.45		40.8
	16.8		40.0
	17.6	09527	34.0
08795:5D4	20.0		36.0
	18.5		35.6
	18.5		34.0
08796:5D4	17.5		32.8
	17.5		33.2
	17.8		33.2
	17.8	09528	32.8
08793:5D4	20.5		31.6
	19.0		32.0
08797:5D4	15.0		32.0
	16.0		30.0
	17.5	09529	37.6
08452:1D10	26.8		40.8
	28.8	09534	35.6
	24.0	09536	35.6
08453:1D10	26.0	09444:6P9	32.8
	26.25		32.8
	26.95		32.0
08744:1D9	22.2		32.8
	23.49		33.6
	24.44	09428:3P10	32.0
	23.17		32.0
			30.4
$\underline{n}$	30		31
$\bar{x}$	21.4		32.5
s^2	17.41		36.77
SD	4.17		6.06
e^2	0.58		1.19
SEM	0.76		1.09

SECTION 4: TABLE I.

CELL WIDTH (μm)

PROXIMAL COLON	DISTAL COLON
4.6	4.8
4.5	3.7
3.8	4.1
4.0	4.2
5.4	2.8
5.8	3.8
4.2	4.5
4.3	4.0
4.9	4.2
5.3	3.8
5.5	5.8
4.5	5.0
3.7	3.5
4.8	4.5
5.5	3.5
5.4	3.5
5.7	2.8
6.9	3.5
5.5	4.7
5.5	3.0
n 20	20
$\bar{x}$ 4.99	3.96
s^2 0.638	0.607
SD 0.799	0.779
e^2 0.319	0.030
SEM 0.179	0.174

SECTION 4: TABLE II.

	x/y	$(x/y)^2$	hμm	Πhx/y + Π/2	x/y	$(x/y)^2$	hμm	Πhx/y + Π/2
	7.2	51.84	0.8	19.66	9.0	81.00	1.15	34.09
	7.0	49.00	0.95	22.46	8.0	64.00	1.3	34.25
	7.0	49.00	1.0	23.57	9.6	92.16	1.15	36.26
	7.8	60.84	1.05	27.31	9.0	81.00	1.0	29.85
	7.0	49.00	0.83	19.82	8.2	67.24	1.0	27.34
	6.6	43.56	0.75	17.12	7.8	60.84	1.15	29.76
	6.4	40.96	0.6	13.64	8.4	70.56	1.35	37.20
	6.4	40.96	0.62	14.04	10.6	112.36	1.05	36.54
	7.8	60.84	0.6	16.27	9.8	96.04	1.0	32.36
					10.0	100.00	1.05	34.56
x	7.02	49.56	0.8	19.32	9.04	82.5	1.120	33.2
n	9	9	9	9	10	10	10	10
s^2	0.274	55.57	0.030	20.80	0.887	296.2	0.157	10.93
SD	0.324	7.454	0.174	4.561	0.942	17.21	0.125	3.306
e^2	0.305	6.174	0.003	2.311	0.089	29.62	0.002	1.093
SEM	0.176	2.484	0.058	1.520	0.298	5.44	0.039	1.045

PROXIMAL COLON, CONTROL

DISTAL COLON, CONTROL

x/y = microvilli/um

$(x/y)^2$ = microvillus density/ sq micron cell surface

h = microvillus height in microns

Πhx/y + Π/2 = calculated microvillus surface area/sq micron luminal cell surface.

SECTION 4: TABLE III

	x/y	$(x/y)^2$	hum	Ilhx/y + Il/2
	6.4	40.96	0.6	13.63
	8.4	70.56	1.1	30.60
	7.8	60.84	0.55	15.05
	8.0	64.00	1.0	26.71
	8.6	73.96	1.0	28.59
	5.4	29.16	0.6	11.75
	7.4	54.76	0.8	20.17
	6.8	46.24	0.6	14.39
	7.2	51.84	0.85	20.80
	6.6	43.56	0.95	21.51
$\bar{x}$	7.26	53.59	0.805	20.30
n	10	10	10	10
s^2	0.978	197.69	0.0419	44.130
SD	0.989	14.06	0.205	6.643
e^2	0.098	19.77	0.004	4.41
SEM	0.313	4.45	0.065	2.10

PROXIMAL COLON, POTASSIUM LOADED

x/y	$(x/y)^2$	hum	Ilhx/y + Il/2
8.8	77.44	1.1	31.99
9.4	88.36	1.05	32.58
10.6	112.36	0.95	33.21
10.0	100.00	0.95	31.42
8.4	70.56	0.85	30.60
8.6	73.96	0.95	27.24
9.4	88.36	0.95	29.63
9.8	96.04	0.93	30.05
10.4	108.16	1.15	37.52
9.6	92.16	1.35	42.49
9.2	84.64	1.05	31.92
9.47	90.20	1.025	32.59
11	11	11	11
0.498	179.75	1.019	16.87
0.706	13.41	0.138	4.108
0.045	16.34	0.002	1.534
0.213	4.04	0.042	1.238

DISTAL COLON, POTASSIUM LOADED

SECTION 4: TABLE III (CONT'D).

STATISTICAL COMPARISON OF MICROVILLUS PARAMETERS USING STUDENT'S T TEST.

Distal colon vs proximal colon	t	∅	p
microvillus density	5.982	17	0.001
microvillus height	4.567	17	0.001
Calculated surface area	7.523	17	0.001
Distal colon vs K distal colon			
microvillus density	1.136	19	NS
microvillus height	1.65	19	NS
calculated surface area	0.376	19	NS
Proximal colon vs K proximal colon			
microvillus density	0.791	17	NS
microvillus height	0.056	17	NS
calculated surface area	0.378	17	NS
Proximal K colon vs distal K colon			
microvillus density	6.092	19	0.001
microvillus height	2.854	19	0.02
calculated surface area	5.041	19	0.001

SECTION 4: TABLE IV.

iii. ULTRASTRUCTURAL STUDY OF LATERAL CELL MEMBRANES.

At a magnification of x10,000 the lateral cell membranes are readily visible. There appears to be an increased complexity at the basal portion of the cell (fig 4.9) with both horizontal and lateral interdigitations. There do not appear to be desmosomes in this region; they are limited to the upper portion of the cell.

Figure 4.10 demonstrates the upper half of the proximal epithelial cells shown in figure 4.9. Again, there are complex interdigitations, with well defined junctional complexes uniting the cells near the luminal surface and frequent desmosomes along the length of the membranes. The 'blurring' of definition in some portions of the micrograph indicates that the intercellular membranes have been sectioned obliquely. Photomicrographs at this magnification were used in the determination of membrane surface concentration. It can be appreciated that it is not possible to include an entire cell in one 8" x 10" micrograph. However at a lower magnification, the definition of the intercellular membranes is inadequate.

In distal colon, the intercellular membranes appear to be interdigitating in a more complex way with several layers of infoldings visible (fig 4.11). The animals which received dietary potassium supplements have more complex lateral cell membranes with an increased amount of both horizontal (fig 4.12) and vertical (figs 4.13,14) infolding. The most striking change is apparent in the distal colon of potassium loaded animals, where there is a marked increase in the complexity of infolding (fig 4.15).

At higher magnification, details of the lateral cell membrane near the luminal surface are demonstrated in figures 4.16 - 4.20. There are no striking differences in these examples between proximal and distal colon of control animals. However, there is an obvious difference between distal (fig 4.19,20) and proximal colon (fig 4.18) in potassium loaded animals.

Based on these subjective impressions, the evidence for significant differ-

ences existing between the experimental groups is not conclusive. It is for this reason that morphometric analysis is necessary to quantify these qualitative differences.

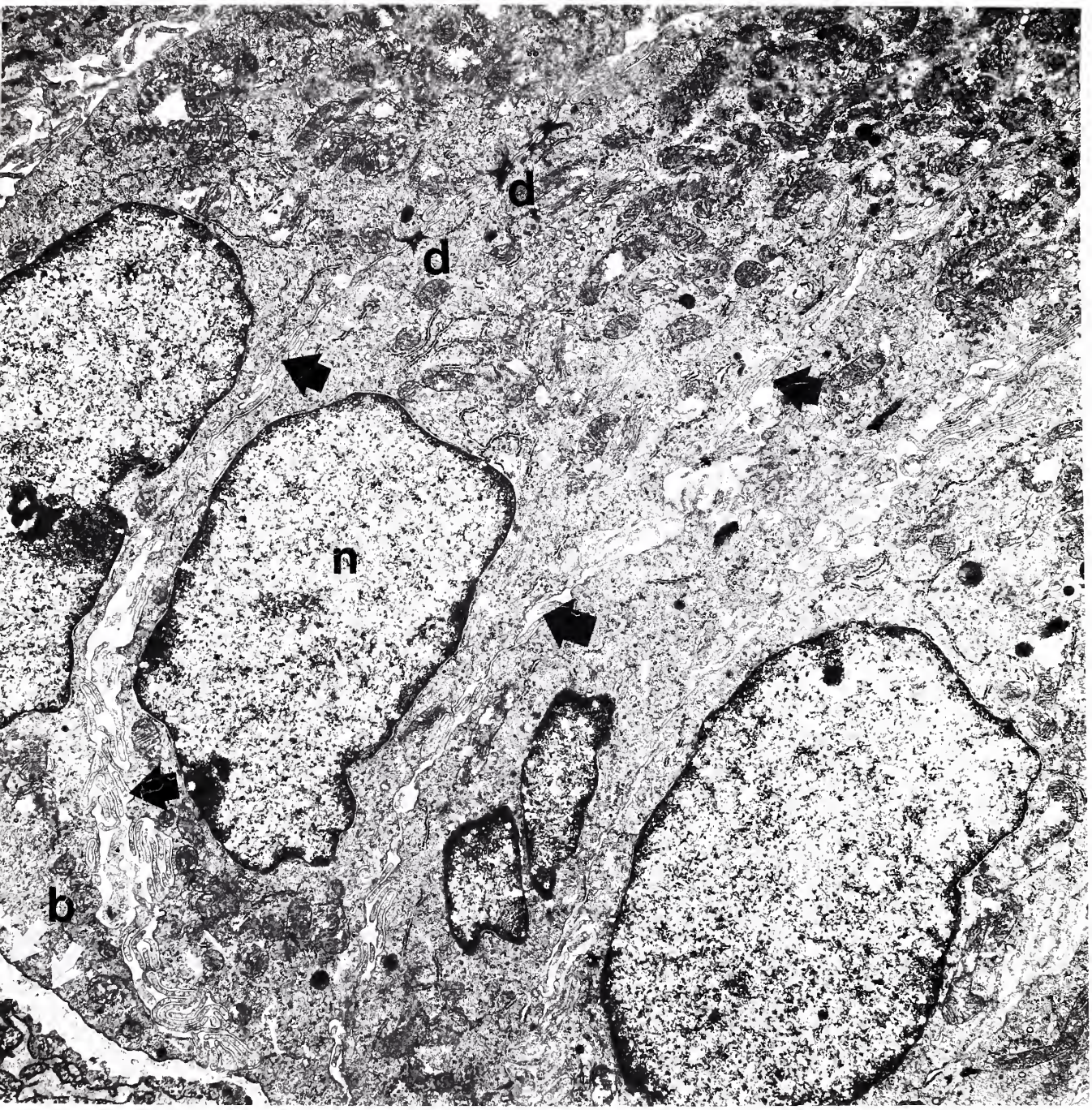


Figure 4.9. Proximal colon, basal portion of cell x10000. Basement membrane (b) baso-lateral membrane (arrowed) nucleus (n) desmosomes (d).

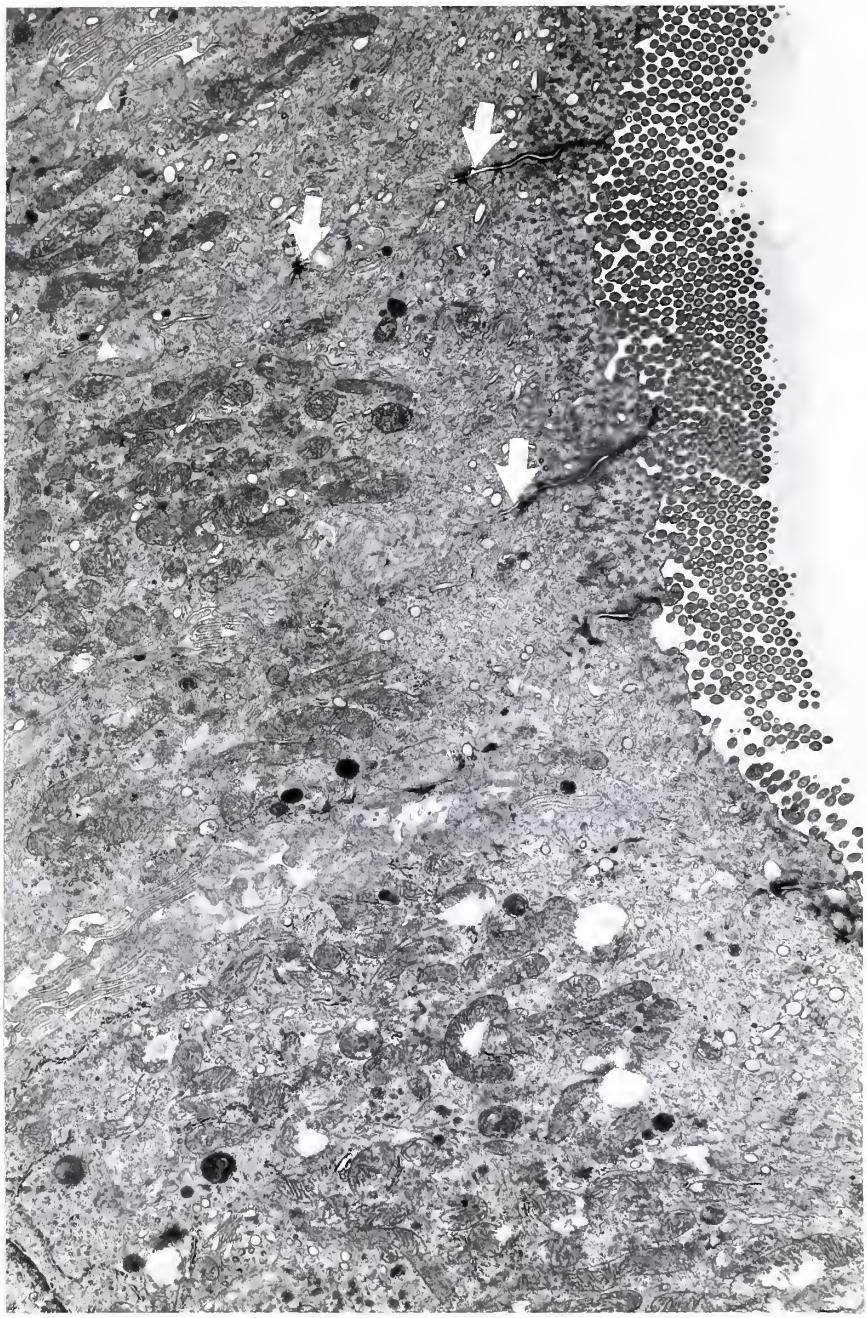


Figure 4.10. Proximal colon, apical portion of cell x10000. Appearance of lateral cell membranes in same cell as previous figure.

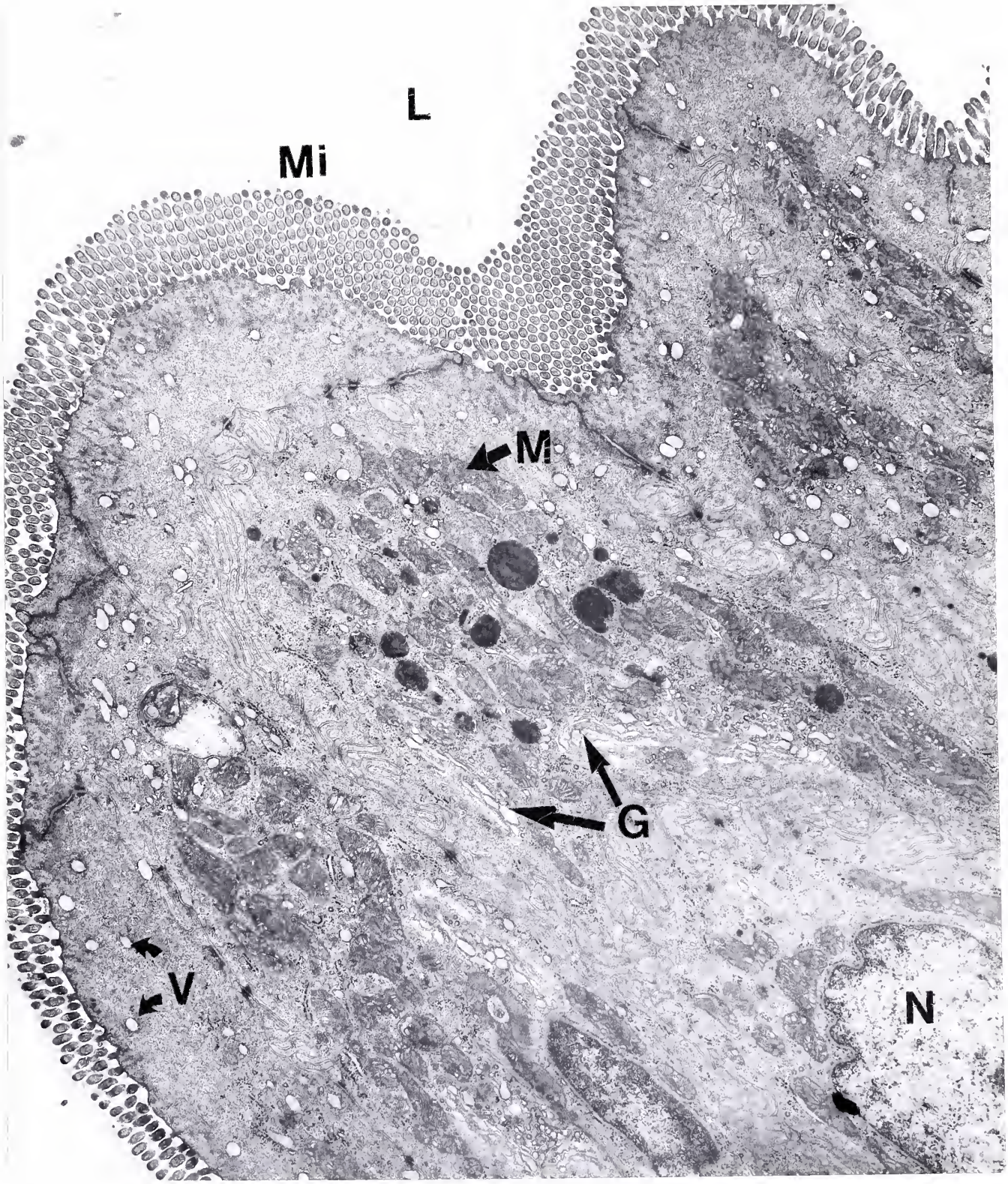


Figure 4.11. Absorptive cells of rat distal colon, x15,750 Microvilli (Mi) gut lumen (L) Golgi apparatus (G) nucleus (N) mitochondria (M) vesicles (v)

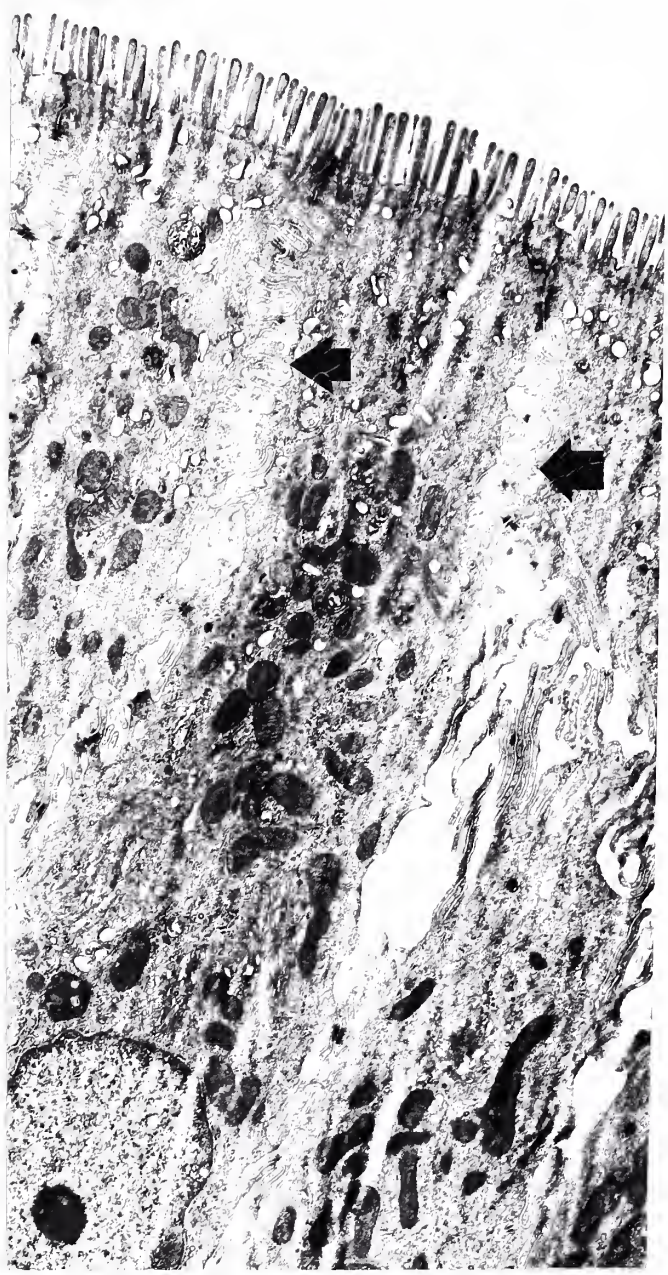


Figure 4.12. Appearance of intercellular membranes of proximal rat colon after potassium loading x8000. Note increased horizontal interdigitation (arrowed) below luminal surface.

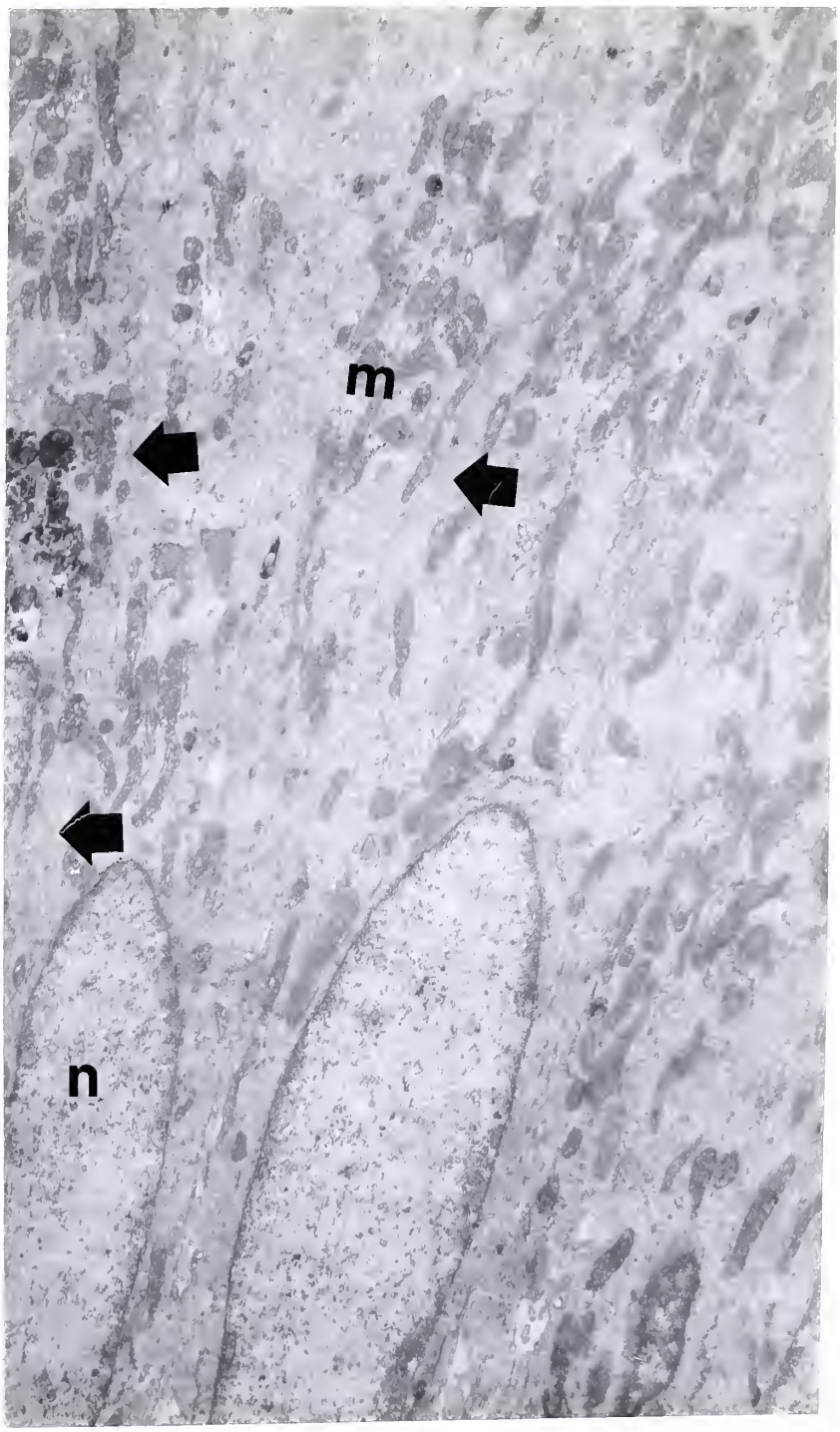


Figure 4.13. Proximal colon, potassium loaded rat x10000. Intercellular membranes showing vertical interdigitations (arrowed) mitochondria (m) nucleus (n).

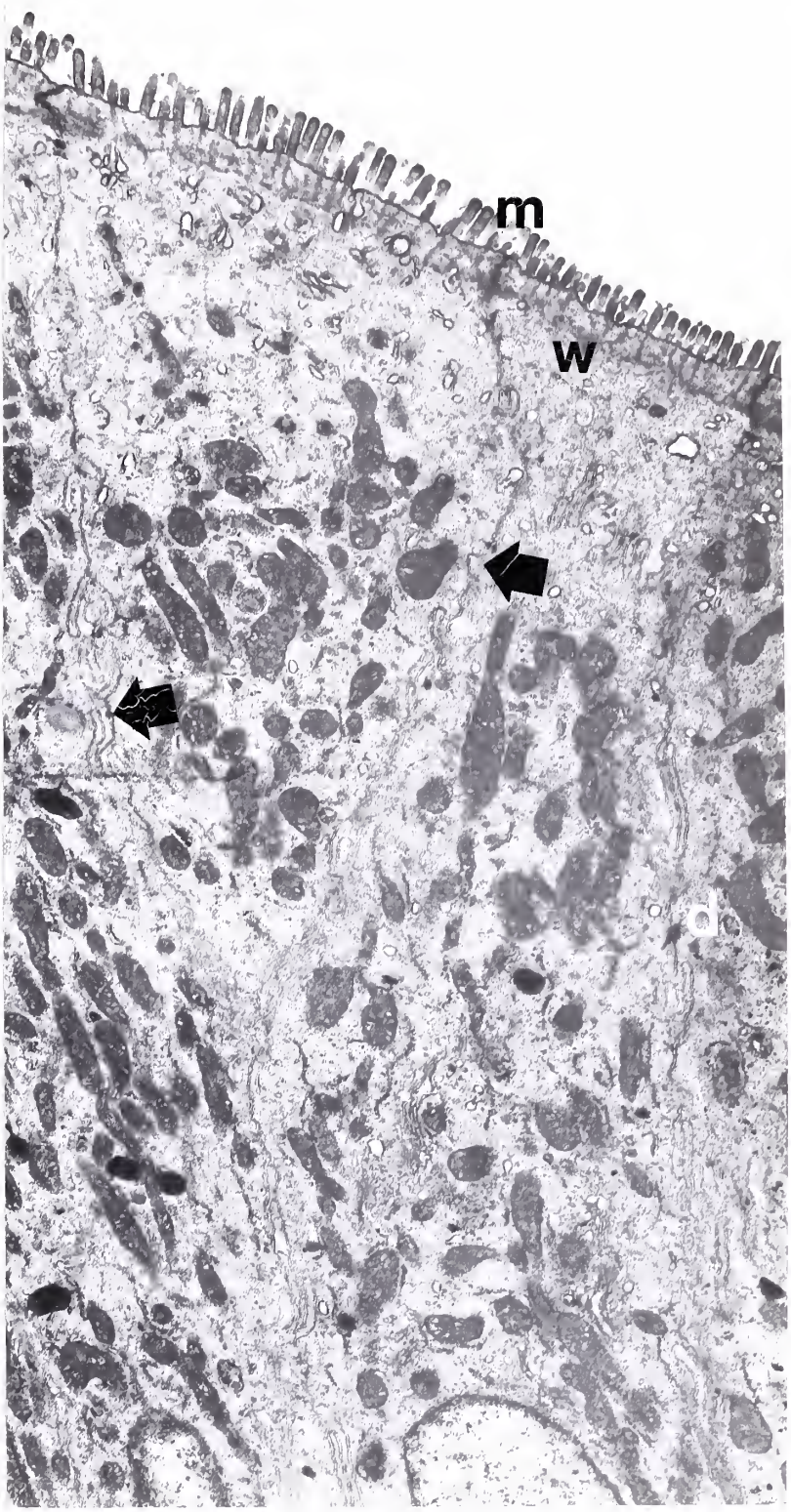


Figure 4.14. Proximal colon, potassium loaded rat x10000. Intercellular membranes (arrowed) microvilli (m) terminal cell web (w) desmosomes (d).

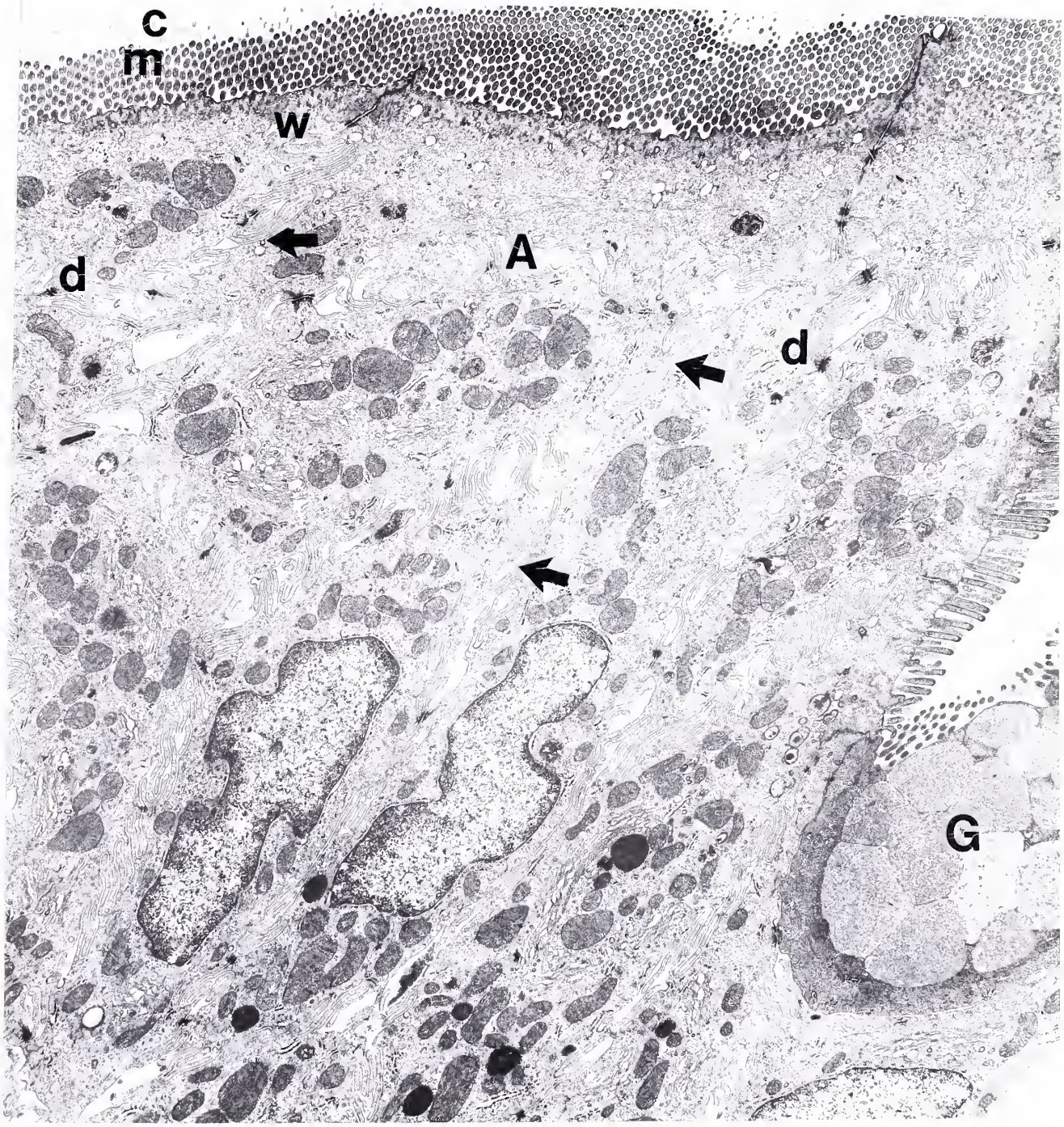


Figure 4.15. Distal colon, potassium loaded rat x10000. Absorptive cells (A) goblet cell(G) in crypt base, microvilli (m) cell coat (c) terminal web (w) desmosomes (d) intercellular membrane (arrowed).

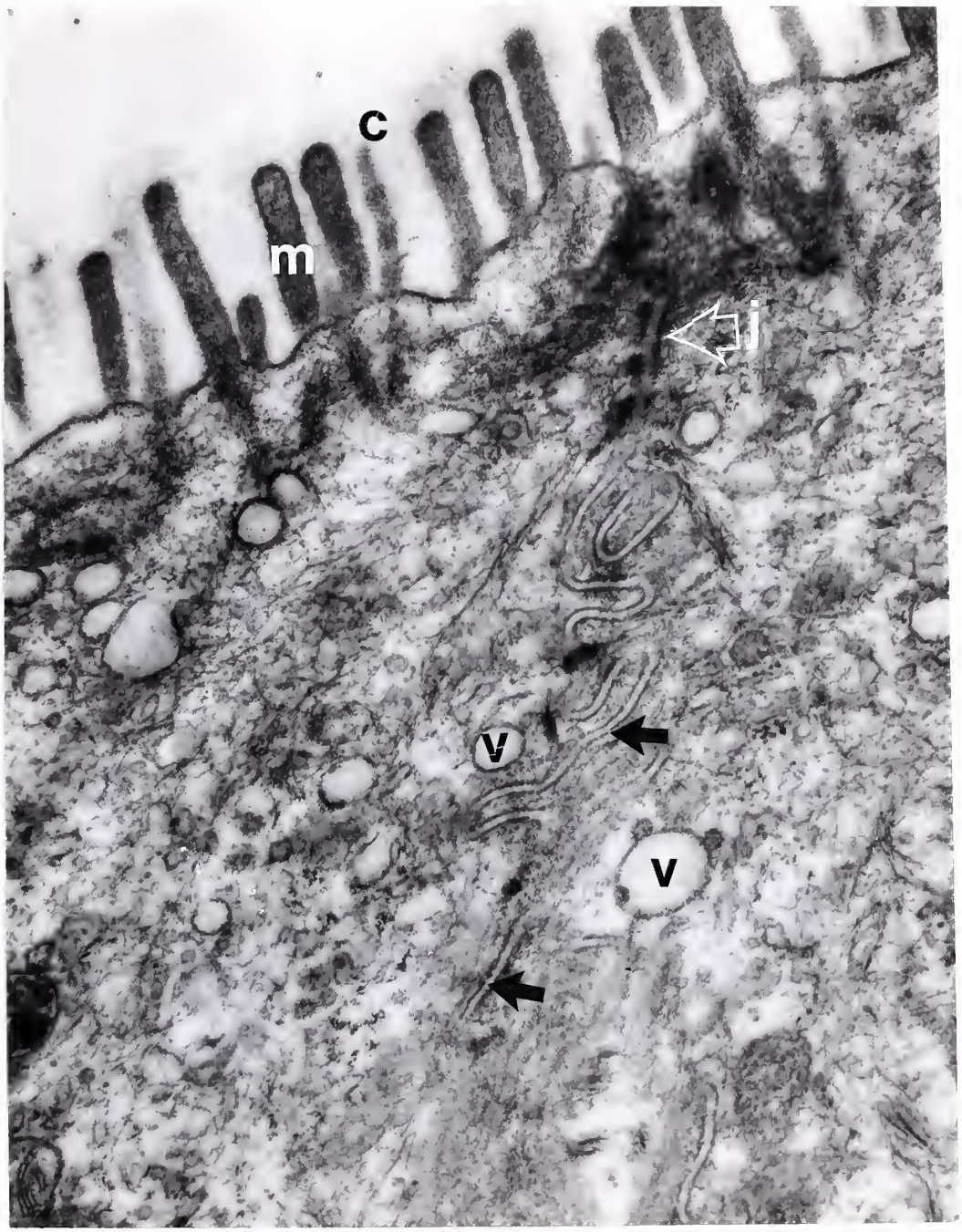


Figure 4.16. Absorptive cells of proximal colon, control x50000. Microvilli (m) cell coat (c) intercellular membranes (arrowed) coated vesicles (v) cell junctional complex (j).

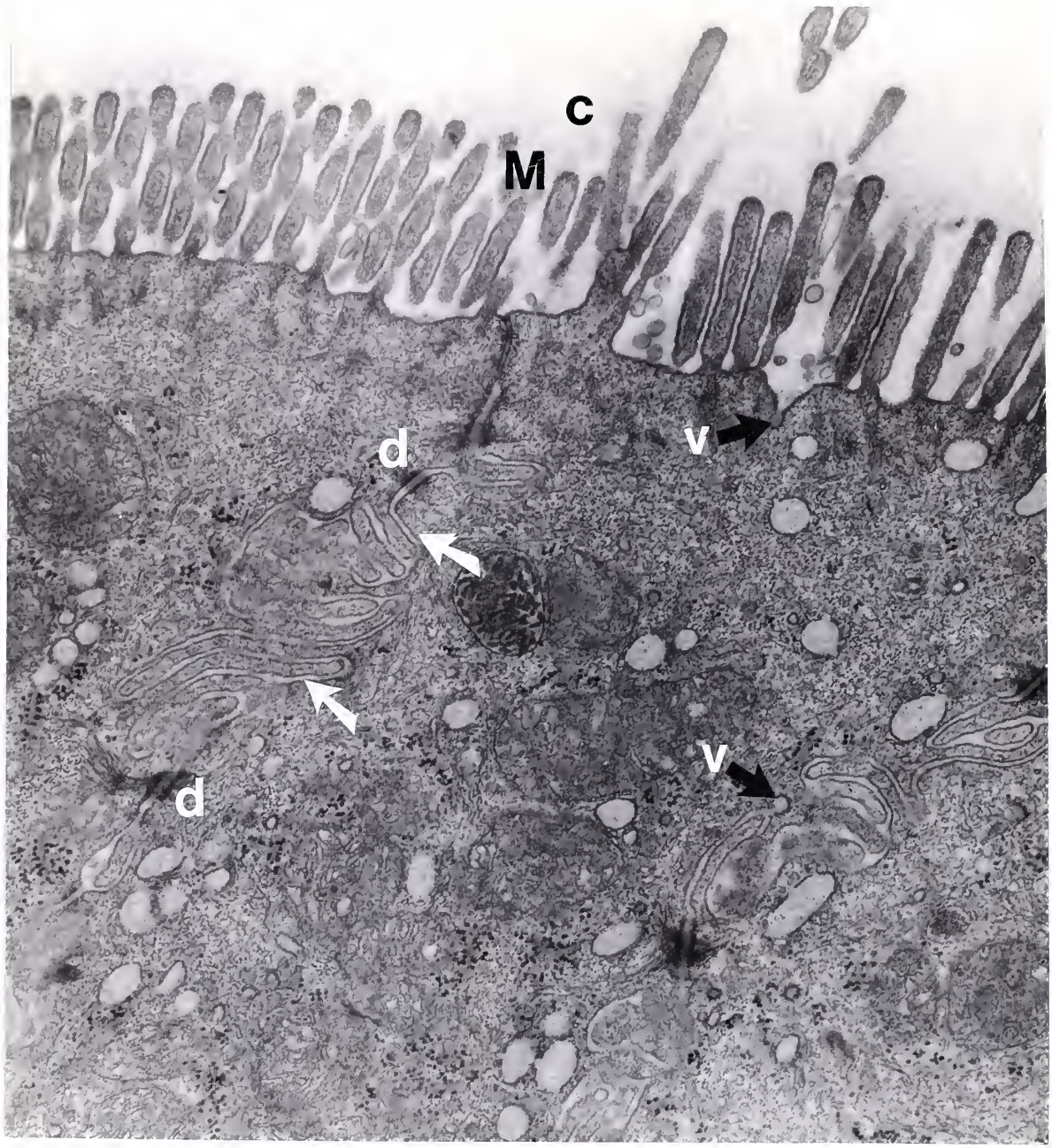


Figure 4.17. Absorptive cell distal colon, control x50000. Microvilli (m) cell coat (c) vesicles (v) fusing with luminal surface and intercellular membrane (arrowed) desmosomes (d).

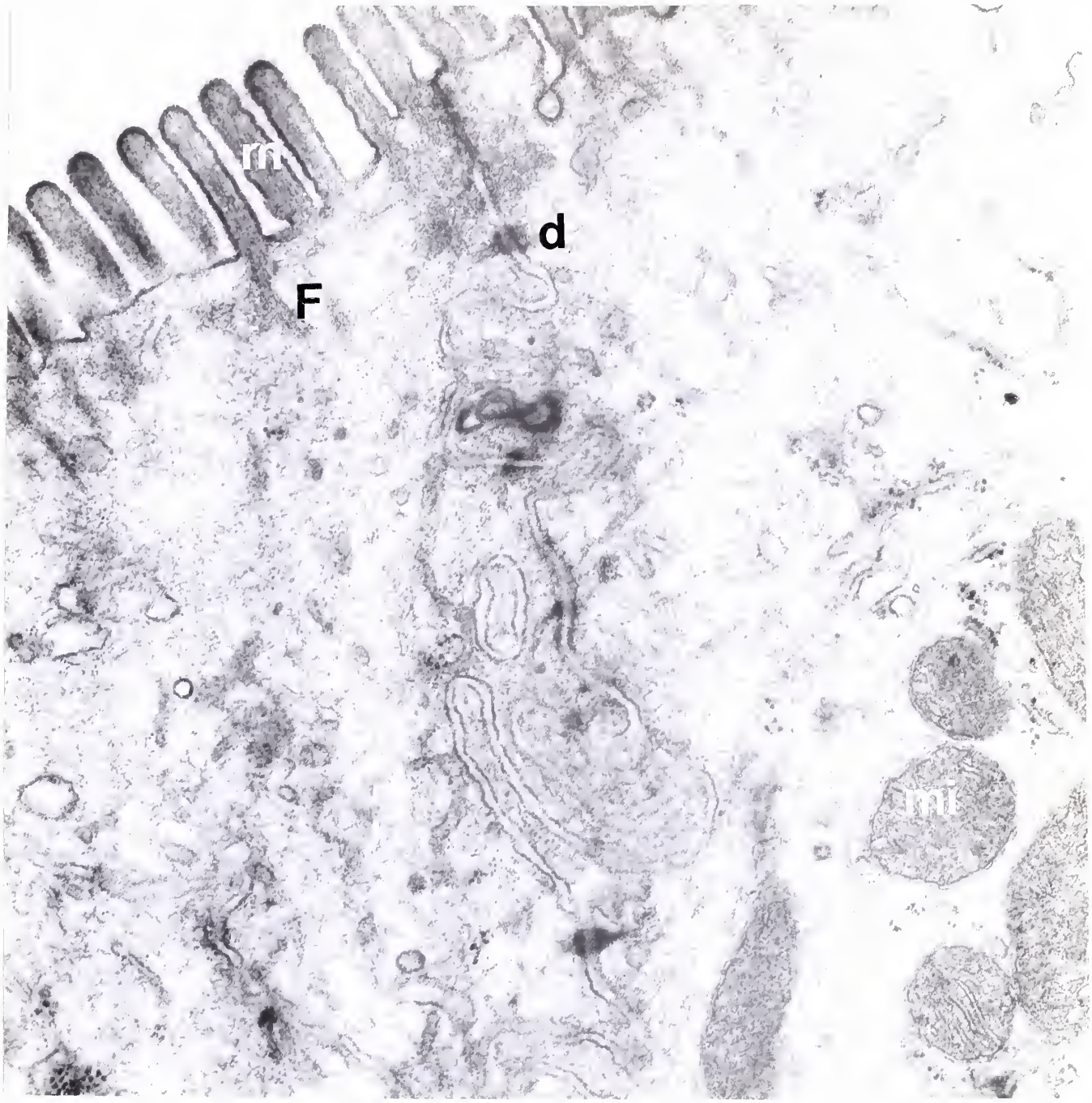


Figure 4.18. Absorptive cells of proximal colon, potassium loaded rat x62500. Microvilli (m) microfilaments (F) desmosomes (d) mitochondria (mi)

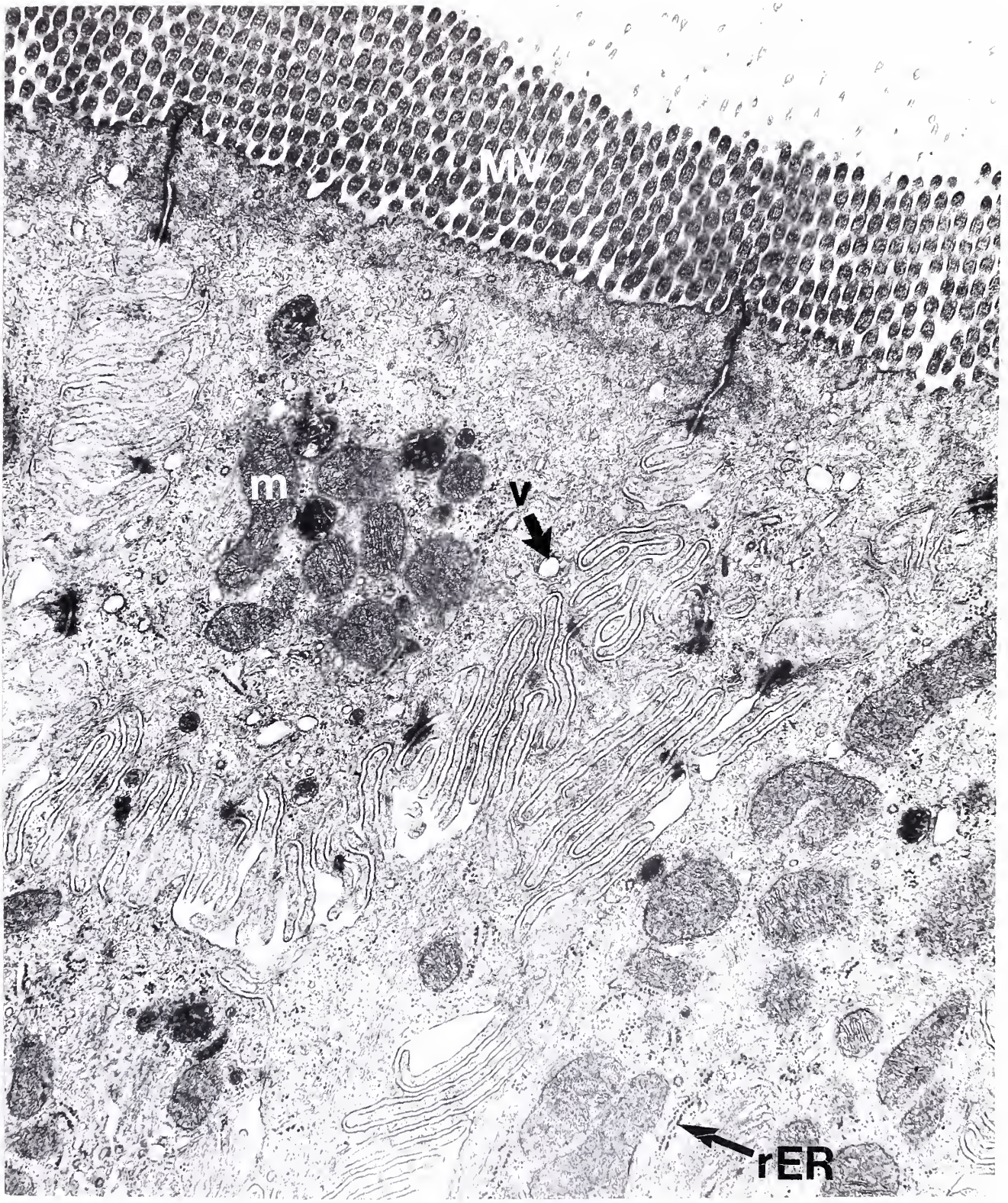


Figure 4.19. Absorptive cells of distal colon, potassium loaded rat x31250. Rough endoplasmic reticulum (rER) mitochondria (m) vesicles (V) microvilli (mv).

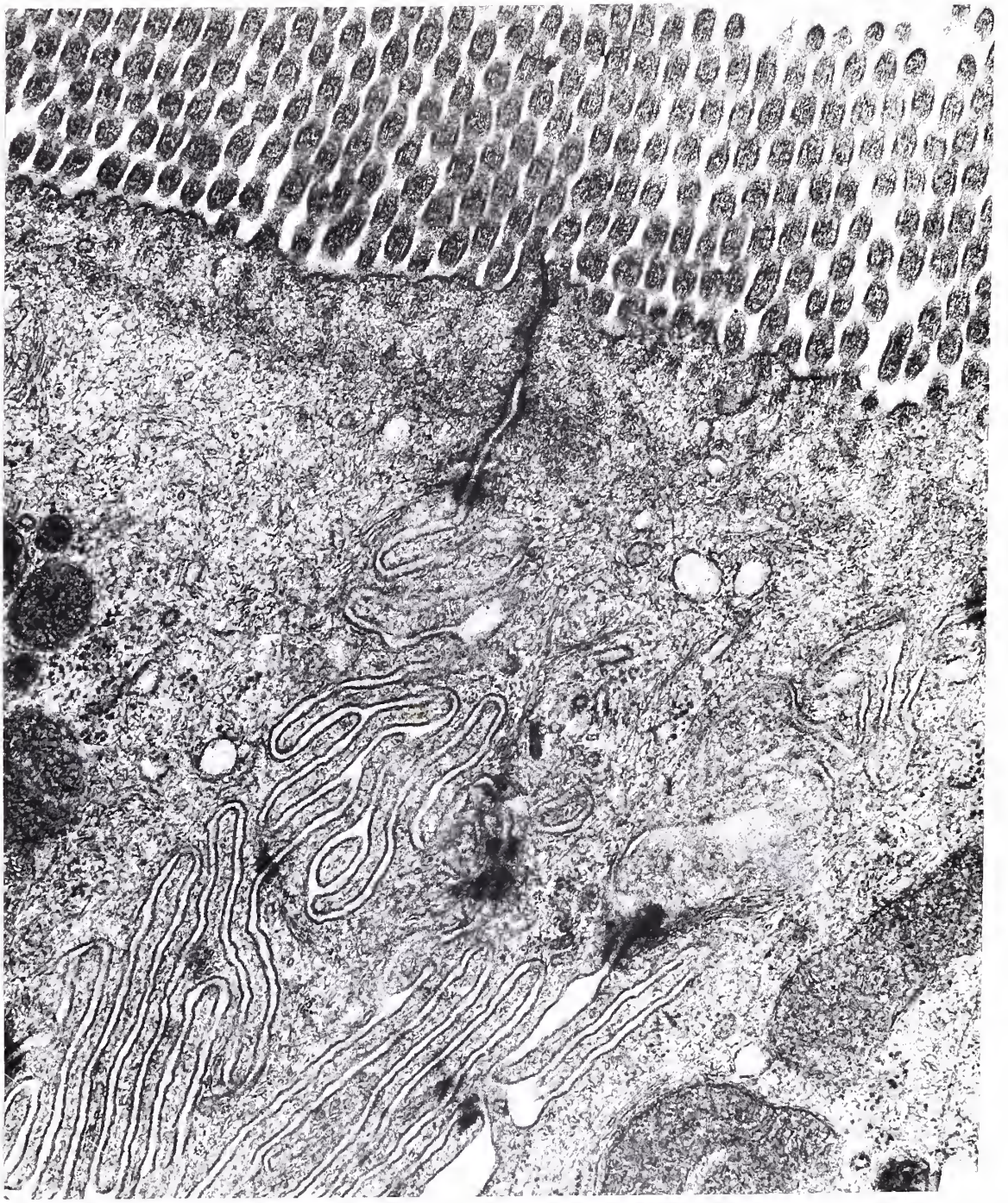


Figure 4.20. Absorptive cells of distal colon in potassium loaded rats x50000.

iv. QUANTITATIVE ESTIMATION OF LATERAL CELL INTERFACES IN PROXIMAL AND DISTAL COLONIC EPITHELIUM

Membrane surface concentration was measured and compared (see Table VI) in proximal and distal epithelial cells. The mean intercellular MSC in proximal colonic epithelium is 2.82 ± 0.118 μ^2/μ^3. SEM is quoted unless otherwise stated. For distal colonic epithelium, the MSC is 3.74 ± 0.173 μ^2/μ^3, $p < .001$. The MSC is thus 32.8% higher in distal colon (Table VII).

QUANTITATIVE ESTIMATION OF INTERCELLULAR MEMBRANES IN PROXIMAL AND DISTAL COLON OF POTASSIUM LOADED RATS

Membrane surface concentration was also measured in colonic epithelial cells of potassium loaded rats (see Table VI). The mean MSC in proximal colon is 3.87 ± 0.173 μ^2/μ^3, and in distal colon, 5.55 ± 0.192 μ^2/μ^3. $p < .001$. MSC in distal colon of potassium loaded rats is thus 43.4% higher. Animals loaded with dietary potassium show a 37% increase in MSC of proximal colon compared with controls ($p < .001$), and a marked increase of 48% in MSC of distal colonic cells compared with controls ($p < .001$).

These results are summarised in Table VIII.

PROXIMAL COLON, CONTROL

CELL	ORIENT-ATION	CROSSINGS (C)	LENGTH (Lmm)	MAGNI-FICATION	MEMBRANE SURFACE CONCENTRATION (CM/250L)	
0963:5P16	0/90	526	7049	10000	2.985	2.818
	45	467			2.650	
09309:5P15	0/90	416	5775	10000	2.881	2.833
	45	402			2.784	
09365:5P16	0/90	941	7702	10000	4.887	4.583
	45	824			4.279	
09312:5P15	0/90	616	7390	10000	3.334	3.578
	45	706			3.821	
09362:5P16	0/90	422	5912	10000	2.855	2.656
	45	363			2.456	
09310:5P15	0/90	489	6550	10000	2.986	3.099
	45	526			3.212	
09459:5P15	0/90	551	7250	10000	3.040	2.899
	45	500			2.759	
09457:5P15	0/90	580	7530	10000	3.081	2.930
	45	523			2.778	
09456:5P15	0/90	573	7173	10000	3.195	3.273
	45	601			3.351	
09458:5P15	0/90	578	7929	10000	2.916	2.490
	45	552			2.785	
08411:4P3	0/90	807	7345	10000	4.395	3.807
	45	591			3.219	
08420:4P3	0/90	559	7843	10000	2.851	2.718
	45	507			2.586	
08421:4P3	0/90	533	8356	10000	2.542	2.559
	45	540			2.576	
08852:4P3	0/90	768	8233	10000	3.731	3.381
	45	624			3.032	
08851:4P3	0/90	482	8325	10000	2.316	2.121
	45	401			1.927	
08425:1P1	0/90	466	7426	10000	2.510	2.510
	45	466			2.510	
08427:1P1	0/90	679	8158	7875	3.329	3.363
	45	693			3.398	
08736:1P5	0/90	493	8178	10000	2.411	2.458
		512			2.504	

PROXIMAL COLON, CONTROL (CONT'D).

CELL	ORIENT-ATION	CROSSINGS	LENGTH	MAGNI-FICATION	MEMBRANE SURFACE CONCENTRATION (CM /250L)	
08731:1P5	0/90	731	7380	10000	3.734	3.377
	45	591			3.019	
08720:1P4	0/90	506	6337	10000	3.194	3.156
	45	494			3.118	
08843:1P5	0/90	479	8165	10000	2.437	2.092
	45	375			1.837	
08842:1P5	0/90	340	8119	10000	1.675	1.734
	45	364			1.793	
08840:1P5	0/90	461	8348	10000	2.209	2.434
	45	555			2.659	
08473:1P5	0/90	341	7777	10000	1.754	1.674
	45	310			1.594	
08747:1P5	0/90	520	8086	10000	2.572	2.419
	45	458			2.266	
08726:1P4	0/90	667	8004	10000	3.333	2.929
	45	505			2.524	
08849:4P3	0/90	456	7776	10000	2.346	2.642
	45	571			2.937	
08821.4P2	0/90	444	7740	10000	2.295	2.300
		446			2.305	

SECTION 4: TABLE VI (CONT'D)

DISTAL COLON, CONTROL.

CELL	ORIENT-ATION	CROSSINGS (C)	LENGTH (L mm)	MAGNIFICATION	MEMBRANE SURFACE CONCENTRATION (CM/250L).	
08454:1D10	0/90	955	7254	10000	5.793	5.632
	45	902			5.741	
08830:1D10	0/90	703	7905	10000	3.557	3.666
	45	746			3.775	
08846:1D9	0/90	388	8387	10000	1.850	1.924
	45	418			1.994	
08829:1D10	0/90	577	8314	10000	2.776	
	45	672			3.233	3.005
08833:1D10	0/90	732	7960	10000	3.678	3.897
	45	819			4.116	
08834:1D10	0/90	694	8174	10000	3.396	3.702
	45	819			4.008	
08772:1D11	0/90	795	7500	10000	4.240	4.134
	45	755			4.027	
08745:1D9	0/90	641	7032	10000	3.646	3.709
	45	663			3.771	
08758:1D11	0/90	638	6820	10000	3.741	3.575
	45	581			3.408	
08760:1D11	0/90	665	7357	10000	3.616	
	45	651			3.539	3.578
08793:5D4	0/90	1201	7796	10000	6.162	5.626
	45	992			5.090	
08795:5D4	0/90	624	5371	10000	4.647	4.427
	45	565			4.208	
09326:5D11	0/90	718	7853	10000	3.657	3.545
	45	674			3.433	
09324:5D11	0/90	424	7680	10000	2.208	2.285
	45	471			2.361	
09328:5D11	0/90	617	7524	10000	3.280	2.988
	45	507			2.695	
09333:5D11	0/90	963	8088	10000	4.763	5.025
	45	1069			5.287	
09327:5D11	0/90	639	7182	10000	3.559	3.392
	45	579			3.225	
09373:4D12	0/90	589	6974	10000	3.378	3.418
	45	603			3.459	
09372 :4D12	0/90	813	7657	10000	4.247	3.539
	45	542			2.831	

TABLE VI (CONT'D)

POTASSIUM LOADED, PROXIMAL COLON

CELL	ORIENT-ATION	CROSSINGS (C)	LENGTH (Lmm)	MAGNIFICATION	MEMBRANE SURFACE CONCENTRATION (CM/250L)	
08783:6P10	0/90	652	7446	10000	3.503	3.282
	45	570			3.062	
08786:6P10	0/90	1012	8385	10000	4.828	4.506
	45	877			4.184	
08784:6P10	0/90	1057	6811	10000	6.208	5.614
	45	855			5.201	
08785:6P10	0/90	742	7098	10000	4.184	4.089
	45	709			3.996	
08782:6P10	0/90	739	6870	10000	4.303	4.157
	45	689			4.012	
09359:6P12	0/90	608	7922	10000	3.070	2.830
	45	513			2.590	
09449:6P9	0/90	919	8512	10000	4.318	4.624
	45	1049			4.930	
09446:6P9	0/90	647	7501	10000	3.450	3.373
	45	618			3.296	
09443:6P9	0/90	454	5632	10000	3.224	3.491
	45	529			3.757	
08805:3P12	0/90	588	7760	10000	3.031	3.307
	45	695			3.582	
08799:3P12	0/90	488	8186	10000	2.385	2.597
	45	575			2.810	
08800:3P12	0/90	830	8549	10000	3.883	4.031
	45	893			4.178	
08853:3P12	0/90	815	7335	10000	4.444	4.935
	45	995			5.426	
08803:3P12	0/90	652	6202	10000	4.205	4.805
	45	838			5.405	
08801:3P12	0/90	807	7172	10000	4.501	4.883
	45	944			5.265	
09337:6P12	0/90	811	8207	10000	3.952	3.616
	45	673			3.280	
09293:3P8	0/90	602	7300	10000	3.299	3.178
	45	558			3.038	

TABLE VI (CONT'D)

PROXIMAL COLON, POTASSIUM LOADED (CONT'D).

CELL	ORIENT-ATION	CROSSINGS (C)	LENGTH (Lmm)	MAGNIFICATION	MEMBRANE SURFACE CONCENTRATION (CM/250L).	
09292:3P8	0/90	658	8084	10000	3.256	3.241
	45	652			3.226	
09429:3P10	0/90	884	8487	10000	4.166	3.916
	45	778			3.667	
09430:3P10	0/90	612	7251	10000	3.376	3.180
	45	541			2.984	
09425:3P10	0/90	675	7471	10000	3.614	3.515
	45	638			3.416	

TABLE VI (CONT'D)

DISTAL COLON, POTASSIUM LOADED

CELL	ORIENT-ATION	CROSSINGS (C)	LENGTH (L mm)	MAGNIFICATION	MEMBRANE SURFACE CONCENTRATION CM/250L.	
08807:3D19	0/90	678	5053	10000	5.525	5.692
	45	740			5.858	
08808:3D19	0/90	893	8214	10000	4.349	4.064
	45	776			3,779	
08806:3D19	0/90	1039	5402	10000	7.693	7.468
	45	978			7.242	
08811:3D17	0/90	708	5069	10000	5.587	5.358
	45	650			5.129	
08814:3D17	0/90	1315	9371	10000	5.613	5.365
	45	1199			5.118	
08788:3D18	0/90	826	6918	10000	4.775	4.651
	45	783			4.527	
08792:3D18	0/90	516	4255	10000	4.850	5.217
	45	594			5.584	
09299:3D19	0/90	805	5310	10000	6.064	6.060
	45	804			6.056	
09432:3D19	0/90	1129	6688	10000	6.752	7.299
	45	1312			7.847	
09436:3D19	0/90	1156	6922	10000	6.680	6.521
	45	1101			6.362	
09439:3D19	0/90	741	5991	10000	4.947	4.900
	45	727			4.854	
08776:6D19	0/90	1174	8148	10000	5.763	5.940
	45	1246			6.117	
08780:6D19	0/90	910	7063	10000	5.154	4.984
	45	850			4.814	
09344:6D15	0/90	944	7138	10000	5.290	5.458
	45	1004			5.626	
09351:6D15	0/90	740	7340	10000	4.033	4.289
	45	834			4.545	
09366:6D19	0/90	1202	7577	10000	6.346	6.404
	45	1224			6.462	
09454:6D19	0/90	873	7147	10000	4.886	5.250
	45	1003			5.614	
09455:6D19	0/90	802	6749	10000	4.753	4.635
	45	762			4.516	

TABLE VI (CONT'D)

DISTAL COLON, POTASSIUM LOADED (CONT'D)

CELL	ORIENT-ATION	CROSSINGS (C)	LENGTH (L mm)	MAGNIFICATION	MEMBRANE SURFACE CONCENTRATION (CM/250L)	
09451:6D19	0/90	957	7943	10000	4.819	4.557
	45	853			4.296	
09367:6D16	0/90	1078	6797	10000	6.344	6.373
	45	1088			6.403	
09349:6D15	0/90	948	7043	10000	5.384	5.733
	45	1071			6.083	
09350:6D15	0/90	1224	8196	10000	5.974	5.771
	45	1141			5.569	

TABLE VI (CONT'D)

MEASUREMENT OF INTERCELLULAR MEMBRANE SURFACE CONCENTRATION

IN CONTROL AND POTASSIUM LOADED RATS.

	Proximal Control	Distal Control	Proximal K loaded	Distal K loaded.
$\bar{x}$	2.82	3.74	3.87	5.55
n	28	19	21	22
s^2	0.392	0.907	0.625	0.812
SD	0.626	0.952	0.791	0.901
e^2	0.014	0.047	0.029	0.037
SEM	0.118	0.219	0.173	0.192

	t	∅	p
Control: distal vs proximal	3.721	45	0.001
K: distal vs proximal	6.5	41	0.001
Control distal vs K distal	6.202	39	0.001
K proximal vs control proximal	5.501	47	0.001

SECTION 4: TABLE VII

SUMMARY OF RESULTS

	PROXIMAL		DISTAL		P (P vs D)
Cell height Hμm	36.8 ± 1.09 (31)		21.4 ± 0.76 (30)		.001
Cell width μm	5.0 ± 0.18 (20)		4.0 ± 0.17 (20)		.001
Microvillus density per μm^2 cell surface					
Control	49.6 ± 2.48 (9)		82.5 ± 5.44 (10)		.001
		NS		NS	
K^+ loaded	53.6 ± 4.45 (10)		90.2 ± 4.04 (11)		.001
Microvillus height (μm)					
Control	0.80 ± 0.06 (9)		1.12 ± 0.04 (10)		.001
		NS		NS	
K^+ loaded	0.81 ± 0.07 (10)		1.03 ± 0.04 (11)		.02
Estimated surface area of microvilli per μm^2 cell surface					
Control	19.3 ± 1.52 (9)		33.2 ± 1.05 (10)		.001
		NS		NS	
K^+ loaded	20.3 ± 2.10 (10)		32.6 ± 1.24 (11)		.001
Membrane surface concentration (μm^2/ μm^3)					
Control	2.8 ± 0.12 (28)		3.7 ± 0.22 (19)		.001
		.001		.001	
K^+ loaded	3.9 ± 0.17 (21)		5.6 ± 0.19 (22)		.001

Note: Figures quoted ± SEM, numbers in parentheses = n

SECTION 4: TABLE VIII

5. DISCUSSION

i.MORPHOLOGIC DIFFERENCES BETWEEN PROXIMAL AND DISTAL COLON

Although colonic ultrastructure has been described in the past, few studies have mentioned differences between cells of proximal and distal colon.As a number of authors have identified regional variation in fluid and electrolyte handling and transmural potential difference, it is of interest to compare the two regions in terms of structure and function

First, a consideration of cell size reveals two distinct populations. The proximal colonic cells sampled are 70% longer and 25% wider than their distal counterparts. Secondly, the proximal colonic absorptive cell nucleus is smooth, round and elongated in comparison to more distally sited nuclei, which are irregular, often with deep invaginations and clefts which are unsuspected when sections are examined under the light microscope. The significance of this is obscure, and operational mechanisms governing nuclear morphology are poorly understood[8]; shape has been related to motility, for example in smooth muscle cells, whose nuclei are smooth and elongated when the cell is relaxed, and ovoid and invaginated when it is contracted. Change in shape also occurs in response to maturation and ageing (in polymorphonuclear leucocytes), and in cells with increased nucleocytoplasmic exchange and heightened metabolic activity (in silk spinning glands, tumors). Of these possibilities, the last may be applicable to distal colonic epithelium. However, differences in cell size and nuclear shape seem to relate to a specific degree of differentiation, and the significance of this is unknown. It would be useful to examine other segments of colon to see if there is a gradual change in morphology consistent with a field theory of differentiation, or whether there is an abrupt transitional zone.

The presence of many mitochondria and a well-developed Golgi apparatus strongly suggest that colonic epithelium is actively engaged in metabolic activity. This has been correlated with size and number of mitochondria and

complexity of cristae, as, for example, in kidney tubules, where energy is needed for active transport across membranes[8]. Colonic epithelium is analogous in this respect.

ii. THE LUMINAL CELL SURFACE.

A further difference between proximal and distal colonic absorptive cells becomes apparent upon consideration of the characteristics of the luminal cell surface. In distal colon, there are not only 66% more microvilli per square micron of luminal cell face, but each microvillus is 40% longer. If the surface area is estimated, this difference in morphology causes an increase of 72% in distal colon when compared to proximal colon.

The functional significance of this is not clear. Obviously the luminal cell surface is the major pathway for fluid and electrolyte exchange, as intercellular pathways make little contribution in colon. The colon is an organ functionally adapted, predominantly, to absorption of water, sodium and chloride, although it secretes bicarbonate and potassium into gut lumen. There are several elements to consider in the characterisation of transport systems which have been discussed previously. First there are two possible transport mechanisms, active transport and passive diffusion. Second is the structure of the epithelium which incorporates a system of two membranes, the luminal and the baso-lateral, across which transport takes place. Is it possible to relate the surface area of the luminal cell membrane to the rate of passive diffusion or to the ionic gradients established across the epithelium measured as transmural potential difference? It is known that the passive permeability to sodium is three times higher in proximal colon than in distal[22], and that although distal colon transports less in absolute terms than proximal colon[40], it develops a higher transmural potential difference.

It would appear, therefore, that the amount of transport across the colon does not depend on the surface area of the interface between gut lumen and

cell, ie the surface area of the luminal cell membrane, as more transport occurs in proximal colon. It is possible that the area of the luminal membrane is a factor in the expression of the transmural PD set up by active transport across the baso-lateral membrane.

Active transport in the colon may depend not only on the activity of the Na-K-ATPase pumps on the baso-lateral membranes, but also upon the gradient set up by discrepancies between luminal and baso-lateral membrane surface areas. In proximal colon, this ratio is estimated as 1:4.75 (L:ICM), and in distal colon, as 1:2.38 (luminal SA to intercellular SA). Thus in proximal colon, although intercellular membrane surface concentration and estimated luminal surface area are less than in distal colon,the ratio between the two is higher.

It is interesting that, in dietary potassium loading, the luminal cell surface area remains virtually constant but the intercellular MSC increases in both proximal and distal colon (vide infra). Thus the ratio of luminal surface area to intercellular surface area increases to 1:6.29 (proximal) and 1:3.68 (distal). This may have a functional significance, but also demonstrates the specificity of the response to potassium loading.

iii. LATERAL CELL MEMBRANES IN NORMAL COLONIC EPITHELIUM.

Although morphometry is a popular technique, no previous studies appear to have been carried out comparing inter-organ variability in colonic absorptive cell lateral cell membranes. It is not known if membrane surface concentration (a measure of lateral cell membrane surface area) is constant during growth and development or under normal physiologic conditions, and how it responds to various hormonal and pharmacological stimuli.

Qualitative increases in intercellular membranes have been described in other tissues known to participate in active transport. For example, following jejunostomy, ileal hypertrophy occurs which is accompanied by an increase of Na-K-ATPase in mucosal homogenates, and increased intercellular membrane

complexity[74]. Saline loading in ducklings produces deep infoldings in the basal and lateral surface of the avian salt gland epithelium[75]. These examples demonstrate that secretory epithelium can change adaptively by increasing the surface area of intercellular membranes.

Subjective changes in cell morphology can be quantified by morphometry; colonic epithelium was chosen for analysis in this study because of increasing interest in the colon as a model for the investigation of ion transport which has analogous properties to distal tubule of kidney.

A significant difference exists in the membrane surface concentration between proximal and distal segments of colon. The values $-2.8 \pm 0.12(SEM)\mu^2/\mu^3$ in proximal colon vs $3.7 \pm 0.22(SEM)\mu m^2/\mu m^3$ in distal colon are baseline measurements against which others can be compared. At present one can only speculate on their significance. It is possible that the difference may be related to the fixation process, if there are different osmolalities in proximal and distal colon under resting conditions. Fixation of the organ at one osmolality might cause differential shrinkage in proximal or distal colon. The surface area of the intercellular membranes would not be affected, but the apparent cell volume and intercellular spaces might change. There would however be a valid basis for comparison between normal and potassium loaded rats providing that the osmolality of the fixative used was kept constant (as was the case).

The difference between proximal and distal cells may be representative of morphologic differentiation, or it may be the response of different target organs to hormonal stimuli. For example, the colon develops a higher potential difference distally after aldosterone infusion[23],and it is possible that aldosterone provokes development of intercellular membranes in colonic epithelium.

The amount of Na-K-ATPase has been shown to fall 30 to 50% following adrenalectomy[25,76,78] and this is accompanied by decreased absorption of sodium and water and decreased potassium secretion. While aldosterone infusion following adrenalectomy has been shown to produce an increase in quantity of Na-K-

ATPase which was significant[78], other authors[77] do not feel that an increase in this enzyme is the initial event in mineralocorticoid induced transport changes in colon, as they showed that infusion of aldosterone for twenty hours produced an increase in PD and short circuit current with no concomitant changes in enzyme activity.

Bastl, Hayslett and Binder[79] showed that PD and sodium absorption in colon were unaltered in adrenalectomised animals infused with aldosterone (10µg/100g) but there was a slight increase in potassium secretion. Thus, although aldosterone has an effect on electrolyte transport, it is not felt to be the primary mediator of changes in absorption and secretion.

The effect of mineralocorticoids, however, does appear to be specific to colonic epithelium. DOCA, which is about 1/30 as potent as aldosterone in its mineralocorticoid effect[80] increases sodium and water absorption, potassium secretion and PD and Na-K-ATPase activity in colon alone, not jejunum or ileum[32]. Recently, it has been demonstrated that glucocorticoids exert a profound effect on water and electrolyte movement in colon. Dexamethasone infusion in rats (600µg/100g) over three days produces an increase in sodium and water absorption and an increase in potassium secretion and PD that is correlated with an increase in Na-K-ATPase activity in colonic mucosal homogenates[31]. Following adrenalectomy, dexamethasone (10µg/100g) restores sodium secretion, potassium absorption and PD to normal[79].

Glucocorticoids may not have an effect specific to colon alone, however, as methylprednisone, a steroid with 1/5 the potency of dexamethasone in its anti-inflammatory effects, but with a small amount of mineralocorticoid activity[80] has been shown to increase sodium and water absorption, potassium secretion, PD and Na-K-ATPase activity in jejunum, ileum and colon[32].

It would be most interesting to examine the relationship of the lateral cell membrane surface area to the amount of Na-K-ATPase activity present - an assay of this enzyme in proximal and distal colon would be a useful correlate

of active transport with the membrane surface concentration. The effect of aldosterone or dexamethasone replacement following adrenalectomy would also be valuable in determining the influence of hormonal factors on cell morphology.

iv. THE ROLE OF THE LATERAL CELL MEMBRANE IN POTASSIUM ADAPTATION IN COLON.

Chronic dietary potassium loading produces a significant increase in colonic epithelial lateral cell membrane surface area, expressed as membrane surface concentration. The response in proximal and distal colon is not the same: whereas in proximal colon the MSC increased 37% to 3.87 ± 0.173 $\mu m^2/\mu m^3$, in distal colon there was a 48% increase to 5.55 ± 0.192 $\mu m^2/\mu m^3$.

Thus a morphologic effect of dietary loading on colonic epithelium has now been demonstrated. This may be correlated with a number of previously documented physiologic effects, namely an increase in Na-K-ATPase activity[78], an increase in transmural PD and potassium secretion, with no alterations in other membrane bound enzymes such as 5' nucleotidase[24]. This is not accompanied by any alteration of sodium, water or chloride transport, and does not occur in jejunum[24], muscle, brain or liver[62].

The phenomenon of potassium adaptation appears to be a response specific to kidney and colon. In kidney, increased Na-K-ATPase activity[59,62], PD[64] also accompany the increase in potassium secretion caused by dietary potassium loading. Partial renal ablation increases the activity of renal Na-K-ATPase and the rate of urinary potassium secretion[59,63]. More recently, it has been demonstrated that with renal insufficiency, the colon also increases the rate of potassium secretion, transmural PD, Na-K-ATPase activity, accompanied by increased sodium absorption[25].

v. MECHANISMS OF POTASSIUM ADAPTATION.

It was initially felt that aldosterone was the mediator of this response; adrenalectomy causes a decrease in Na-K-ATPase activity and a 50% drop in colonic transmural PD[78] and a loss of response to dietary potassium loading. Aldosterone partially restores this response, but another mineralocorticoid, DOCA

(50µg/day IM) alone had no effect. Potassium can stimulate the production of aldosterone[81] and since both aldosterone and potassium produce an increase in transmural PD and Na-K-ATPase activity in ascending and descending colon, it is possible that aldosterone mediates potassium adaptation in part at least.

Aldosterone is a 21-C mineralocorticoid secreted by the zona glomerulosa of the adrenal cortex: the target organs for this hormone include such specialised epithelium as salivary glands, distal renal tubule, colon and toad bladder[82]. It is presumed to act through RNA transcription to promote synthesis of a specific protein and has a rate-limiting role in the absorption of sodium ions, either by increasing the cell's ability to pump sodium serosally, by activating Na-K-ATPase or increasing the amount of energy available to drive the pump, or by increasing the permeability of the luminal epithelium[80]. Recently it has been shown that, in toad bladder epithelium, aldosterone increases uptake of precursors into mRNA and is inhibited by spironolactone[83].

In similar experimental models, however, to that of Silva et al[78], Fisher et al[24] fed adrenalectomised rats a high potassium diet; Na-K-ATPase levels rose to pre-adrenalectomy levels and transmural PD increased to normal, suggesting that potassium adaptation cannot be mediated by aldosterone alone. This effect is analogous to that of distal renal tubule, where Na-K-ATPase activity increases in response to dietary potassium loading even after adrenalectomy[59, 62,63]. However, aldosterone does cause an increase in Na-K-ATPase activity in renal cortex[65].

There are several ways in which an increase in dietary potassium might directly influence colonic epithelium. It is possible that epithelial cells adapted to potassium secretion are sensitive to an increase in whole body potassium, or to minute changes in serum potassium concentration[25]. Although some authors noted no alteration in serum potassium during their experiments on colonic adaptation in renal insufficiency[25], or using potassium enriched diets[81], small but significant increases have been noted in animals on a high

potassium diet during nocturnal feeding hours[84]. Certainly, in chronically depleted animals, where serum potassium concentration falls to 2.5mEq/l from a mean of 3.6mEq/l, net potassium secretion in perfused colon falls to zero[37].

Schon et al[63] suggest that in kidney, an increase in potassium secretion occurs in response to an increased load of potassium per unit nephron, with increased uptake by the Na-K-ATPase pump, rise in the intracellular potassium pool and increased movement of potassium passively across the luminal membrane. Tracer studies have demonstrated an increase in the transport pool of potassium in the distal tubule of rats fed a high-K diet, and a fall in animals on a low-K diet[85]. This mechanism could also explain increased potassium secretion in colon.

Potassium secretion in the normal rat colon may be described by existing electrochemical gradients ascribed to the active absorption of sodium[36,37,46], although debate about an active component continues[40,48]. Potassium secretion is not dependent on flow rate in colon lumen[24,25], but appears to have a direct linear relationship to transmural PD; this may well enhance the force driving cellular potassium ions passively across the luminal membrane[24,25]. However, it is of interest that acute intravenous infusion of potassium in animals with increased Na-K-ATPase activity secondary to renal insufficiency has been shown to result in increased renal clearance of potassium[63] and increased colonic secretion of potassium[24,25] without change in colonic PD.

In the chronically potassium adapted rat, several studies[24,25,78] have linked an increase in potassium secretion with an increase in Na-K-ATPase activity in colon, as they have in distal renal tubule[59,62]. When potassium chloride is infused acutely into animals with renal insufficiency, colonic potassium secretion is three times higher than in controls[25]. It is reasonable to link the increased Na-K-ATPase activity causally with this event.

In potassium adaptation in colon, the increase in Na-K-ATPase activity is not associated with changes in sodium transport[24]. Na-K-ATPase activity can

also increase in the absence of changes in potassium secretion, but with increased sodium secretion and transmural PD in hypertrophied rat cecum[86]. It is likely therefore that this enzyme can function selectively in its uptake of ions, and that potassium transport in chronic potassium adaptation in colon is not sodium coupled, as in, for example, red cells and brain[87].

Can the changes in lateral cell membranes documented in this study be correlated in some way with the increased potassium secretion, PD and Na-K-ATPase activity previously shown to accompany chronic dietary potassium loading? There are a number of ways in which an increase in lateral membrane surface area could lead to an increase in potassium secretion, by increasing the area available for passive potassium transport, altering cell resistance, or by influencing active transport in some way.

Ninety per cent of cell potassium exchanges across the baso-lateral membranes in descending colon[88]. It is possible that an increase in the intercellular membrane is associated with an increase of potassium entry into the cell: although no channels have been identified in the serosal membranes which permit potassium entry, potassium may enter the cell this way, even if it is coupled to the Na-K-ATPase pump. Potassium accumulation in the cell is dependent on active transport[34].

Evidence to date has not suggested that potassium is secreted via an inter cellular route, although it may diffuse back from lumen to interstitial fluid via paracellular and cellular pathways. Perhaps an increase in length of the intercellular channel or an increase in the membrane resistance might retard back diffusion of potassium and thus contribute to an increase in net secretion.

The affinity of colonic Na-K-ATPase for potassium does not appear to change as in potassium loaded animals, the K_m remains constant but V_{max} increases. This suggests that the amount of potassium transport across the baso-lateral membrane stimulates an increase in the number of Na-K-ATPase linked pumps[24].

Na-K-ATPase has been demonstrated ultrastructurally in rabbit renal medulla[89]. It appears exclusively in membrane form, in ultra-thin sections as flat

or cup-shaped triple-layered membrane fragments with a diameter of 0.1 to 0.6μm. Particles 30 to 50Å diameter span the membrane and are seen on inner and outer surfaces. There are 12.500 ± 2500 (SD) per μm^2 and two surface particles unite to form a unit of Na-K-ATPase that binds one molecule of ATP or ouabain.

It is therefore possible that tha intercellular membranes expand in area to accommodate an increased number of Na-K-ATPase units, which are well defined space occupying complexes in the plasma membrane. Na-K-ATPase in colon is located in the baso-lateral membranes[55] and the lateral cell membrane surface area was found to increase in this study with dietary potassium loading. It is significant that this adaptive response appears to be limited to the serosal cell surface - measurements of microvillus density and luminal cell surface area estimates were not shown to differ significantly from control values. Microvilli are thought to be stable differentiations of the cell surface whose primary function is absorption[8], but in certain circumstances they are capable of adaptation. For example, hypertrophy has been described in ileum following jejunostomy[74]. Microvilli are certainly susceptible to damage - following de-oxycholate infusion in colon microvilli are shortened and lost[90].

Thus the response which has been characterised in this study is specific to the intercellular colonic membrane and so there is an evident association with previously documented increases in Na-K-ATPase in the baso-lateral membrane. One can only speculate over whether these facts are associated cause and effect. It would be most useful to analyse colonic epithelium to determine the distribution and density of the Na-K-ATPase units, in addition to determining enzyme activity in control and potassium loaded rats. Also, the proximal and distal colon should be analysed separately - the quantitatively different response to potassium loading, and the well defined anatomical differences between the two regions indicate that proximal and

distal colon are capable of responding differently physiologically. Evidence reviewed here suggests that the distal colon is capable of creating larger ionic gradients, and it can develop a larger transmural PD in response to aldosterone and DOC[23,30]. This holds true also in potassium loaded rats[24]. It is important that quantitative assays of Na-K-ATPase activity are made in potassium loaded animals, making a distinction between proximal and distal colon.

Singer and Nicolson's fluid mosaic model of cell membrane structure is still currently accepted[91]: this provides for protein mobility within the membrane, including lateral mobility. The charge on the membrane proteins is one of the factors limiting their distribution. It is possible that the Na-K-ATPase units exist at a specific density in the baso-lateral colonic membranes, and that as synthesis of new units occurs, the membrane surface expands in surface area in order to accommodate them. There may be synthesis not only of Na-K-ATPase units but also of the supporting membranes.

It is interesting that potassium has such a profound effect on cell metabolism, whether it is mediated by aldosterone or other hormones, or directly. Silva, Brown, and Epstein[92] have noted that, in potassium loaded kidneys, Na-K-ATPase activity takes three or four days to increase, suggesting a series of metabolic steps including the formation of new protein. This ultrastructural study in colon certainly provides morphologic evidence of increased protein synthesis in an analogous model; it must be inferred from the presence of increased lateral cell membrane density in the potassium loaded rats.

Monovalent cations appear to have profound, direct effects on cell activity. The fate of potassium within the cell is largely unknown - it may form a salt with a phosphorylated carbohydrate, but it probably exists mostly free in solution[93]. Studies made on epithelial cells attempted to determine the distribution of intracellular sodium and potassium, which may be unevenly distributed in intracellular compartments. Palmer and Civan[94] noted that in Chironomus salivary gland cells, electro-chemical activities for sodium, chloride, and potassium, and their electric potentials, were the same within as outside the

cell nucleus. They felt that the endoplasmic reticulum and mitochondria might be responsible for sub-cellular compartmentalisation. Indeed, potassium deficiency produces structural and functional deficits in mamalian ribosomes[95].

These examples provide evidence indicating that potassium has important effects within the cell: it has also been shown to activate several enzymes such as pyruvate kinase by an interaction with substrate and enzyme to produce a ternary complex associated with a conformational change in protein[96]. Interestingly, high concentrations of potassium have been shown in tissue culture of rat adrenal zona glomerulosa cells to cause an increase in the secretion of aldosterone and 18-OH corticosterone, while low concentrations cause an increase in corticosterone and 18-OH DOC secretion. The glomerulosa cell appears directly sensitive to intracellular potassium and the control point may well involve protein synthesis. It is felt that intracellular potassium maintains the activity of the final enzymes involved in the production of aldosterone from DOC[97]. Potassium in fact not only increases aldosterone production, but can also increase insulin secretion which is associated with promotion of potassium transfer intracellularly[93].

This evidence gives weight to the hypothesis that, given the important role of potassium intracellularly, and the necessity of maintaining potassium homeostasis via specific adaptation of certain epithelial cells associated with induction of Na-K-ATPase, it is unlikely that potassium secretion in colon, as in distal renal tubule, is a function of simple diffusion.

6. SUMMARY AND CONCLUSIONS.

This study has demonstrated that morphological differences exist between the absorptive cells of proximla and distal colon in the rat. Quantitative differences also exist in cell size, microvillus density and height and estimated luminal surface area, and in the lateral cell membrane surface area in the cells from these two regions. In the potassium loaded animal, there is a significant increase in the intercellular membrane surface concentration which is most marked distally. No quantitative differences were noted in the number of microvilli per square micron of luminal cell surface, or in microvillus size.

It is concluded that the morphometric analysis of lateral cell membranes in colon may aid in the elucidation of the mechanisms of electrolyte transport. The specific increase in lateral cell membrane surface area in response to chronic dietary potassium loading may be correlated with previously demonstrated increases in transmural potential difference, potassium secretion, and increased Na-K-ATPase activity. Mechanisms whereby the changes in cell membrane concentration might be correlated with these events has been discussed. It is clear that potassium exerts a profound influence on cell metabolism to create this morphologic effect.

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